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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371 INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE DESIGNATED 245-55928 US APPLICATION NO (IF KNOW) OP 1673	763						
CONCERNING A FILING UNDER 35 U.S.C. § 371 OP/673	763						
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INTERNATIONAL ADDITIONAL ADDITIONAL INTERNATIONAL ADDITIONAL ADDIT	MED						
DOTATION (SOLAR)							
PCT/US99/08744 20 April 1999 20 April 1998	(0.00						
TITLE OF INVENTION CHLAMYDIA PROTEINS AND THEIR USES	OIFE						
APPLICANT(S) FOR DO/EO/US	/						
Rockey and Bannantine	OCT 1 6 2000						
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information	E						
1. This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.	PRESENTE TRADEMA						
2. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371	1,7,1						
3. This express request to begin national examination procedures (35 U.S C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C § 371(b) and PCT Articles 22 and 39(1)							
4. A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date							
5. A copy of the International Application as filed (35 U S.C. § 371(c)(2))	5. A copy of the International Application as filed (35 U S.C. § 371(c)(2))						
a 🛛 is transmitted herewith (required only if not transmitted by the International Bureau).							
b has been transmitted by the International Bureau.							
c is not required, as the application was filed in the United States Receiving Office (RO/US)	c is not required, as the application was filed in the United States Receiving Office (RO/US)						
6. A translation of the International Application into English (35 U S.C. § 371(c)(2))							
7. Amendments to the claims of the International Application under PCT Article 19 (35 U S.C. § 371(c)(3))							
a are transmitted herewith (required only if not transmitted by the International Bureau).							
b. have been transmitted by the International Bureau							
c have not been made; however, the time limit for making such amendments has NOT expired.							
d. 🖂 have not been made and will not be made							
	8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).						
9. An oath or declaration of the inventor(s) (35 U.S C. § 371(c)(4)).							
10 A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S C § 371(c)(5))							
Items 11. to 16. below concern document(s) or information included:							
11. An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98, including copies of references cited in International Search Report.							
12 An assignment document for recording. A separate cover sheet in compliance with 37 C F R. §§ 3.28 and 3 31 is included							
13. A FIRST preliminary amendment.							
☐ A SECOND or SUBSEQUENT preliminary amendment							
14. A substitute specification 24197							
15. A change of power of attorney and/or address letter.							
16. Other items or information.							
☑ Written Opinion.							
☐ Preliminary Examination Report. ☐ Computer Readable Form of Sequence Listing.							
☑ International Search Report ☑ Statement in Compliance with 37 C F R §1.821(f)							

532 Rec'd PCT/PTC 16 OCT 2000

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U.S. APPOODON NO JIEKA	owingee (7 (5) \$95)	PCT/US99/08744	TION NO	Į.	RNEY'S DOCKET 1 -55928	NUMBER
17. 🔀 The following fo	ees are submitted:			CAL	CULATIONS	(PTO USE ONLY)
BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):						
Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO						
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO						
International Preliminary Examination fee (37 C.F.R § 1.482) not paid to USPTO but International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO\$710.00						
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)						
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)						
	ENTER APPR	ROPRIATE BASIC	FEE AMOUNT =	\$	690.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than \(\begin{array}{c} 20 \\ \ext{30} \\ months from the earliest claimed priority date (37 C.F.R. \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \				\$	0,000	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			
Total claims	18 - 20 =	0	x \$18.00	\$	0.00	
Independent Claims	7 - 3 =	4	x \$80.00	\$	320.00	
MULTIPLE DEPENI	DENT CLAIM(S) (if appli	icable)	+ \$270.00	\$		
TOTAL OF ABOVE CALCULATIONS =					1010.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed. (Note 37 C.F R. § 1.9, 1.27, 1.28)				\$	101000	
SUBTOTAL =					1010.00	
Processing fee of \$130.00 for furnishing the English translation later than \(\) 20 \(\) 30 Months from the earliest claimed priority date (37 C.F.R. §§ 1.492(f)). +						
TOTAL NATIONAL FEE =				\$_	1010.00	
Fee for recording the enclosed assignment (37 C.F.R. § 1.21(h)). The assignment must be				\$	40.00	
Accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). \$40.00 per property. + TOTAL FEES ENCLOSED =				S	1050.00	
			ES ELICEIOSEE		REFUND →	
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 a. ☐ A check in the amount of \$ 1050.00 to cover the above fees is enclosed. b. ☐ Please charge my Deposit Account No in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed. c. ☐ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4550 A duplicate copy of this sheet is enclosed NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) 						
must be filed and granted to restore the application to pending status.						
SEND ALL CORRESPONDENCE TO: SIGNATURE						
KLARQUIST SPARKMAN CAMPBELL LEIGH & WHINSTON, LLP One World Trade Center, Suite 1600 121 S.W. Salmon Street Tanya M. Harding, Ph.D. NAME						
Portland, OR	397204-2988		42,630 REGISTRATION	N NUM	1BER	

Express Mail Label No. EL696104876US Date of Deposit: October 16, 2000

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Rockey and Bannantine

Art Unit: Not yet assigned

Application No.: Not yet assigned

CERTIFICATE OF MAILING

Filed: Herewith

For: CHLAMYDIA PROTEINS AND THEIR USES

Examiner: Not yet assigned

Date: October 16, 2000

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on October 16, 2000 via Express Mail in an envelope addressed to: BOX PATENT APPLICATION COMMISSIONER FOR PATENTS, WASHINGTON,

D.C. 20231.

Tanya M. Harding Ph.D. Attorney for Applicant

BOX PATENT APPLICATION COMMISSIONER FOR PATENTS WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

Please enter the following amendment in the above-referenced patent application:

In the Specification:

At page 9, line 35, please replace the term "DH5 \square " with --DH5 α --.

At page 12, line 12, please replace the term "DH5 \square " with --DH5 α --.

At page 13, line 35, please replace the term "DH5 \square " with --DH5 α --.

At page 25, please insert the following complete citation after line 8: --Bannanitne. J.P., et al. (1998) Infect. Immun. 66:6017-6021.--.

REMARKS

By this voluntary amendment, the Specification of the application has been amended solely to correct administrative and typographical errors and omissions. The E. coli strain DH5 α is well known, and the corrections to this term are made only to correct a printing error. Support for the insertion of the Bannantine et al. (1998) citation can be found at page

PATENT

14, line 17, which provides sufficient information to locate this article. Applicants believe this amendment adds no new matter to the application.

Respectfully submitted,

KLARQUIST SPARKMAN CAMPBELL LEIGH & WHINSTON, LLP

Tanya M. Harding, Ph.D. Registration No. 42,630

One World Trade Center, Suite 1600

121 S.W. Salmon Street Portland, Oregon 97204

Telephone: (503) 226-7391 Facsimile: (503) 228-9446

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CHLAMYDIA PROTEINS AND THEIR USES

I. FIELD OF THE INVENTION

The present invention relates to the detection of *Chlamydia* and to the diagnosis, treatment and prevention of *Chlamydia* infections in animals.

II. BACKGROUND

Chlamydiae are obligate intracellular bacterial pathogens with a unique biphasic life cycle. They appear as two distinct cellular types, a small dense cell or elementary body (EB) that is enclosed in a rigid bacterial cell wall, and a larger metabolically active reticulate body (RB). The EB is resistant to physical disruption and is infectious, whereas the RB is more fragile and only exists inside cells. The Chlamydia life cycle begins with the attachment of the EB form to the host cell which is followed by endocytosis into a nascent vacuole, also called an "inclusion membrane." After EB attachment and entry, replication of the EB form produces RB forms that continue to grow within the vacuole. By 72 hour post-infection, this growth phase is terminated when the RBs condense, and reorganize back to EBs. The lysis of the host cell results in release of EBs to infect new host cells. The difficulties in working with Chlamydiae center on the obligate intracellular requirement for growth and the fact that no adequate genetic engineering methods have been developed for this organism.

The genus *Chlamydia* includes two species that are primarily associated with human disease: *C. trachomatis* and *C. pneumoniae*. *C. trachomatis* causes trachoma, an eye disease that is the leading cause of preventable infectious blindness worldwide with an estimated 500 million cases of active trachoma worldwide. *C. trachomatis* also causes a sexually transmitted chlamydial disease which is very common worldwide. *C. trachomatis* also causes lymphogranuloma venereum, a debilitating systemic disease characterized by lymphatic gland swelling. The most serious sequelae of chlamydial genital infections of females include salpingitis, pelvic inflammatory disease, and ectopic pregnancy. In the US alone, it is estimated that over 4 million new sexually transmitted *C. trachomatis* infections occurred in 1990, leading to over four billion dollars in direct and indirect medical expenses. The World Health Organization estimates that 89 million new cases of genital *Chlamydia* occurred worldwide in 1995 (Peeling and Brunham, 1996).

C. pneumoniae causes respiratory diseases including so called walking pneumonia, a low-grade disease such that the infected person frequently fails to obtain treatment and remains in the community as an active, infectious carrier. C. pneumoniae is currently of interest because of its strong epidemiological association with coronary artery disease, and there is also some evidence to link it with multiple sclerosis.

Of the other disease-causing species of *Chlamydia*, *Chlamydia psittaci* and *Chlamydia pecorum* are primarily pathogens of wild and domestic animals, but these species may infect

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humans accidentally. *C. psittaci* is acquired through respiratory droplet infection and is considered an occupational health hazard for bird fanciers and poultry workers.

There is tremendous interest in the identification of candidate antigens for protection against chlamydial disease. While a prior infection with *C. trachomatis* will protect against a subsequent challenge by the same strain, indicating a protective component that stimulates the host immune response, most serious chlamydial diseases are exacerbated by an overaggressive anti-chlamydial immune response. Antigens recognized in the context of an infection appear to elicit a protective response whereas immunization with purified, killed (EB form) *Chlamydia* results in an immunopathological response. Therefore for the purposes of vaccine development, one needs to find epitopes that confer protection, but do not contribute to pathology. It is an object of this invention to provide *Chlamydia* polypeptides for use as vaccines that induce a protective immune response without inducing the pathological response caused by the antigens associated with the EB form of *Chlamydia*. Such immunostimulatory peptides will be useful in the treatment, as well as in the diagnosis, detection and prevention of Chlamydial infections.

III. SUMMARY OF THE INVENTION

The present invention includes the use of *Chlamydia* proteins that show enhanced expression in the reticulate body (RB) stage relative to the elementary body (EB) stage of the *Chlamydia* life cycle. These proteins are not present at detectable levels in the EB form using current immunological techniques and are thus said to be "infection-specific." Certain of these infection-specific proteins are found in the inclusion membrane of the infected cell, and so have been termed "Inc" proteins. These include the IncA, IncB, and IncC proteins of *Chlamydia* as described in the present disclosure. The genes that encode the IncA, IncB and IncC proteins are referred to as *incA*, *incB* and *incC* respectively. Other proteins of *Chlamydia* described herein have also been shown by the inventors to be infection-specific, but are not known to be incorporated into the inclusion membrane; these include the p242, TroA, and TroB proteins. The TroA and TroB proteins have been so named because they resemble the Tro proteins of *Treponema pallidum*, which are thought to form part of an ABC transport system.

The inventors have shown that the infection-specific *Chlamydia* proteins of the disclosure are recognized by convalescent antisera (i.e., antisera taken from an animal that has recovered from a *Chlamydia* infection) but are not recognized by antisera against the killed EB form of *Chlamydia*. Thus, the proteins are expressed only during active chlamydial infection and are therefore useful as protective antigens. These infection-specific proteins may be used to confer a protective immune response without inducing a pathological effect. Additionally, immuno-fluorescence microscopy and immunoblotting with antisera demonstrated that the infection-specific proteins are present in *Chlamydia*-infected HeLa cells, but are undetectable in purified EBs and absent in uninfected HeLa cells.

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Immunofluorescense microscopy reveals that IncA, IncB and IncC are localized to the inclusion membrane of infected HeLa cells. Reverse-transcription polymerase chain reactions (RT-PCR), northern hybridization data, and restriction analysis revealed that the *incB* and *incC* genes are closely linked and transcribed in an operon. RT-PCR, restriction analysis and sequential Southern hybridizations of *incA* then *incC* to the same filter provided evidence that *incA* is separated from the *incB* and *incC* operon by about 110 kb. The *C. trachomatis Tro* genes are not closely linked with the p242 gene.

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length. They may even encompass the entire protein.

More specifically, the present invention encompasses the purified infection-specific proteins having amino acid sequences as shown in SEQ ID NOS: 2, 4, 6, 10, and 12, amino acid sequences that differ from such sequences by one or more conservative amino acid substitutions, and amino acid sequences that show at least 75% sequence identity with such amino acid sequences.

Then invention also includes isolated nucleic acid molecules that encode a protein as described in the above paragraph, including isolated nucleic acid molecules with nucleotide sequences as shown in SEQ ID NOS: 1,3, 5, 9, and 11.

The present invention also includes a vaccine or immunostimulatory preparation directed against the reticulate body (RB) form of *Chlamydia* comprising one or more purified infection-specific peptides (or portions or fragments thereof, or peptides showing sequence similarity to a portion of such a peptide). Such peptide fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, of the sequence shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18. Peptides used in such a vaccine may even encompass the entire purified peptide of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or 18, a peptide that differs from such a peptide by one or more conservative amino acid substitutions, or a peptide having at least 75% sequence identity with such a peptide. Such vaccine preparations may contain one or more pharmaceutically acceptable excipients, adjuvants or diluents.

The invention additionally encompasses methods for making a vaccine, comprising combining a pharmaceutically acceptable excipient with a peptide described herein. Also included is a method of vaccination comprising administering a vaccine as described herein to a mammal.

The present invention also provides a method for the diagnostic use of the disclosed purified infection-specific peptides, for instance by use in a diagnostic assay to detect the presence of infection-specific antibodies in a medical specimen, in which antibodies bind to the *Chlamydia* peptide and indicate that the subject from which the specimen was removed was previously

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exposed to *Chlamydia*. Such a method may comprise: (i) supplying a biological sample, such as blood from an animal, that is suspected to contain infection-specific anti-*Chlamydia* antibody, (ii) contacting the sample with at least one infection-specific *Chlamydia* peptide described herein, such that a reaction between the peptide and the infection-specific anti-*Chlamydia* antibody gives rise to a detectable effect, such as a chromogenic conversion; and (iii) detecting this detectable effect.

The present invention also provides a method of using antibodies that bind specifically with the disclosed proteins for detection of infection-specific *Chlamydia* antigen, indicating the presence of *Chlamydia* in the RB stage as distinct from the EB stage. For instance, the relevant infection-specific antibodies may be used to provide specific binding in an Enzyme Linked Immunosorbant Assay (ELISA) or other immunological assay wherein the antibody F_c portion is linked to a chromogenic, fluorescent or radioactive molecule and the F_{ab} portion specifically interacts with, and binds to, an infection-specific protein. Such a method may comprise: (i) supplying a biological sample from an animal suspected to contain an infection-specific *Chlamydia* antigen, and (ii) contacting the sample with at least one infection-specific anti-*Chlamydia* antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect; and (iii) detecting this detectable effect.

Other aspects of the present invention include the use of probes and primers derived from the nucleotide sequences that encode infection-specific peptides, to detect the presence of *Chlamydia* nucleic acids in medical specimens. Such probes and primers may be nucleotide fragments, of, for example, 15, 20, 25, 30 or 40 contiguous nucleotides of the sequence shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17.

An additional aspect of the invention is a method of treating a *Chlamydia* infection by directing a therapeutic agent against a specific target, where the target is chosen from an infection specific protein of *Chlamydia*, a gene that encodes an infection-specific protein of *Chlamydia*, and an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein the therapeutic agent interacts with said target to affect a reduction in pathology.

These and other aspects of the invention will become more apparent from the following description.

30 IV. SEQUENCE LISTING

SEQ ID NO:1 shows a nucleic acid sequence encoding the p242 *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:2 shows the amino acid sequence of the p242 C. trachomatis protein.

SEQ ID NO:3 shows a nucleic acid sequence encoding the TroA *C. trachomatis* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:4 shows the amino acid sequence of the TroA C. trachomatis protein.

SEQ ID NO:5 shows a nucleic acid sequence encoding the TroB C. trachomatis protein, with deduced primary amino acid sequence also shown.

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SEQ ID NO:6 shows the amino acid sequence of the TroB C. trachomatis protein.

SEQ ID NO:7 shows a nucleic acid sequence encoding the IncA *C. psittaci* protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:8 shows the amino acid sequence of the IncA C. psittaci protein.

SEQ ID NO:9 shows a nucleic acid sequence encoding the IncB C. psittaci protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:10 shows the amino acid sequence of the IncB C. psittaci protein.

SEQ ID NO:11 shows a nucleic acid sequence encoding the IncC C. psittaci protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:12 shows the amino acid sequence of the IncC C. psittaci protein.

SEQ ID NO:13 shows a nucleic acid sequence encoding the IncA C. trachomatis protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:14 shows the amino acid sequence of the IncA C. trachomatis protein.

SEQ ID NO:15 shows a nucleic acid sequence encoding the IncB C. trachomatis protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:16 shows the amino acid sequence of the IncB C. trachomatis protein.

SEQ ID NO:17 shows a nucleic acid sequence encoding the IncC C. trachomatis protein, with deduced primary amino acid sequence also shown.

SEQ ID NO:18 shows the amino acid sequence of the IncC C. trachomatis protein.

SEQ ID NO:19 shows the upstream oligonucleotide used to amplify the *C. psittaci inc*C ORF.

SEQ ID NO:20 shows the downstream oligonucleotide used to amplify the *C. psittaci inc*C ORF.

SEQ ID NO:21 shows the upstream oligonucleotide used to amplify the *C. psittaci inc*B ORF.

SEQ ID NO:22 shows the downstream oligonucleotide used to amplify the *C. psittaci* incB ORF.

SEQ ID NO:23 shows the upstream oligonucleotide used to amplify the *C. psittaci inc*A ORF.

SEQ ID NO:24 shows the downstream oligonucleotide used to amplify the *C. psittaci* incA ORF.

V. <u>DESCRIPTION OF THE INVENTION</u>

A. **DEFINITIONS**

Particular terms and phrases used herein have the meanings set forth below.

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"EB" refers to the Elementary Body, an environmentally refractile and largely metabolically dormant form of *Chlamydia* that is infectious and is presented as a small dense body enclosed by a bacterial cell wall.

"RB" refers to the Reticulate Body, a metabolically active form of *Chlamydia* that is not infectious, and exists only within a host cell, being very fragile, often branched, and appearing larger and less dense that the EB.

"Infection-specific" refers to a protein that shows enhanced expression in the RB form of *Chlamydia* compared to the EB form. Infection-specific proteins are not necessarily absent from the EB form, but they are significantly more common in the RB form than in the EB form.

"infection-specific antibody" is an antibody that binds specifically to an infection-specific protein.

"Biological sample" refers to any sample of biological origin including, but not limited to a blood sample, a plasma sample, a mucosal smear or a tissue sample.

"Isolated" An isolated nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

"Probes" and "primers." Nucleic acid probes and primers may readily be prepared based on the nucleic acid sequences provided by this invention. A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, typically DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Probes and primers as used in the present invention typically comprise at least 15 nucleotides of the nucleic acid sequences that are shown to encode infection-specific proteins. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30 or 40 consecutive nucleotides of the disclosed nucleic acid sequences.

Methods for preparing and using probes and primers are well known in the art and are described in, for example Sambrook et al. (1989); Ausubel et al., (1987); and Innis et al., (1990). PCR primer pairs can be derived from a known sequence, for example, by using computer

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programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

"Conservative amino acid substitutions" are those substitutions that, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions.

Original Residue	Residue Conservative Substitution			
Ala	Ser			
Arg	Lys			
Asn	gln, his			
Asp	Glu			
Cys	Ser			
Gln	Asn			
Glu	Asp			
Gly	Pro			
His	asn, gln			
Ile	leu, val			
Leu	ile, val			
Lys	arg, gln, glu			
Met	leu, ile			
Phe	met, leu, tyr			
Ser	Thr			
Thr	Ser			
Trp	Tyr			
Tyr	trp, phe			
Val	ile, leu			

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

"Sequence identity" The similarity between two nucleic acid sequences, or two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Variants of naturally occurring infection-specific peptides useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the amino acid sequence of a

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naturally occurring infection-specific peptide when aligned using BLAST 2.0.1 (Altschul et al., 1997). For comparisons of amino acid sequences of greater than about 30 amino acids, the BLAST 2 analysis is employed using the blastp program set to default perameters (open gap = 11, extension gap = 1 penalty, gap x dropoff = 50, expect = 10, word size = 3, filter on), and using the default BLOSUM62 matrix (gap existence cost = 11, per residue gap cost = 1, lambda ratio = 0.85). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix (gap existence cost = 9, per residue gap cost = 1, lambda ratio = 0.87). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method. such as at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity. The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at http://www.ncbi.nlm.nih.gov/BLAST/. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast help.html.

Similarly, when comparing nucleotides, blastn may be used with default settings (rewards for match = 1, penalty for mismatch = -2, open gap = 5, extension gap = 2 penalty, gap x dropoff = 50, expect = 10, word size = 11, filter on), with the default BLOSUM62 matrix (as above). Variants of naturally occurring infection-specific nucleic acid sequences useful in the present invention are typically characterized by possession of at least 50% sequence identity counted over the full length alignment with the nucleic acid sequence of a naturally occurring infection-specific ORF when aligned using BLAST 2.0.1. Useful nucleic acids may show even greater percentage identity, and may, for example, possess at least 55%, at least 65%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity naturally occurring infection-specific ORF.

"Operably linked" A first nucleic acid sequence is "operably" linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Recombinant" A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

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"Stringent Conditions" Stringent conditions, in the context of nucleic acid hybridization, are sequence-dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5 degrees to 20 degrees lower than the thermal melting point (*Tm*) for the specific sequence at a defined ionic strength and pH. The *Tm* is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989), pages 9.49-9.55. Typical high stringency hybridization conditions (using radiolabeled probes to hybridize to nucleic acids immobilized on a nitrocellulose filter) may include, for example, wash conditions of 0.1 X SSC, 0.5% SDS at a wash temperature of 68°C.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridizes under high-stringency conditions substantially only to the target sequence in a given sample comprising the target sequence.

"Purified" A purified peptide is a peptide that has been extracted from the cellular environment and separated from substantially all other cellular peptides. As used herein, the term peptide includes peptides, polypeptides and proteins. In certain embodiments, a purified peptide is a preparation in which the subject peptide comprises 50% or more of the protein content of the preparation. For certain uses, such as vaccine preparations, even greater purity may be preferable.

"Immunostimulatory peptide" as used herein refers to a peptide that is capable of stimulating a humoral or antibody-mediated immune response when inoculated into an animal.

"Vaccine" A vaccine is a composition containing at least one immunostimulatory peptide which may be inoculated into an animal with the intention of producing a protective immunological reaction against a certain antigen. The antigen to be protected against may be, for instance, an infectio-specific antigen of *Chlamydia*.

B. ISOLATION OF INFECTION SPECIFIC CHLAMYDIA POLPEPTIDES AND IDENTIFICATION OF GENES ENCODING THESE POLYPEPTIDES

1. <u>ISOLATION OF IncA, IncB AND IncC</u>

Bacterial strains. Chlamydia (C. psittaci strain GPIC or C. trachomatis LGV-434, ser. L2) was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified Chlamydiae were obtained using Renografin (E. R. Squibb & Sons, Inc., Princeton, N.J.) density gradient centrifugation. Escherichia coli DH50 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. E. coli XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage vector. E. coli SOLR (Stratagene) was used as the host strain for infection with in vivo excised filamentous lambda ZAPII.

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Antisera. MBP (Maltose Binding Protein)-Inc fusion proteins were used as antigens for the production of mono-specific antibody reagents in Hartley strain guinea-pigs. The protein was diluted to 100 µg/ml⁻¹ sterile saline and mixed with the Ribi Trivalent Adjuvant (Ribi Immunochem.). The antigen/adjuvant emulsion was administered to anaesthetized guinea-pigs using a procedure provided by the manufacturer. Sera were collected 14 days after secondary and tertiary immunizations. Control antisera were produced by immunizing guinea-pigs with adjuvant alone, or with adjuvant plus purified maltose-binding protein.

Convalescent guinea-pig antisera, antisera against live EBs, and antisera against formalinfixed EBs were produced using standard methods (Rockey and Rosquist, 1994 and Rockey et al., 1995).

C. psittaci library construction and screening. For the incB and incC genes, C. psittaci strain GPIC DNA was extracted using a genomic DNA extraction kit (Qiagen) with one modification; dithiothreitol (5mM) was added to the suspension buffer to assist EB lysis. DNA was partially digested with Tsp509I and ligated to EcoRI digested λ-ZAPII phage arms (Stratagene). The ligation was packaged in vitro with Gigapack extracts according to the manufacturer's instructions (Stratagene). Recombinant phage were plated on E. coli XL-1 Blue at densities of approximately 10⁴ PFU/150-mm (diameter) plate. Following a nine hour incubation to allow development of the plaques, the plates were sequentially overlaid with nitrocellulose disks and the resulting lifts were processed for immunoblotting with convalescent antisera and antisera to fixed EBs. Of approximately 8,000 plaques, 18 had reactivity with the convalescent sera but not sera generated against EBs. One of these was subcloned into pBluescript SK(-) phagmid by in vitro excision in the E. coli SOLR strain (Stratagene) and designated pBS200-7.

For the *inc*A gene, genomic DNA from *C. psittaci* strain GPIC was partially digested with *Sau*3A, size-selected (2-8 kb) by electrophoresis through low-melting-temperature agarose, and blunt-ended with T4 DNA polymerase. This DNA was ligated to an *Eco*R1/*Not*1 adapter (Life Technologies), kinased, and ligated to *Eco*R1-digested Lambda ZAP II vector (Stratagene Cloning Systems). Recombinants were packaged (Lambda Gigapack Gold, Stratagene) and used to infect *E. coli* XL1-Blue (Stratagene). Plaques were allowed to develop for 4 h at 37°C. Nitrocellulose filters laden with 10 mM IPTG (US Biochemical Corp.) were placed onto the plaques and incubated for an additional 4 h at 37°C. These filters were removed and placed into a blocking solution consisting of PBS (150 mM NaCl, 10 mM NaPO4, pH7.2) plus 0.1% Tween-20 (TPBS) and 2% BSA-TPBS. Filters were incubated for 1 h, rinsed twice in TPBS, and incubated overnight in convalescent-guinea-pig sera diluted 1:100 in BSA-TPBS. After three washes in TPBS, the filters were incubated for 1 h in ¹²⁵1-staphylococcal protein A (New England Nuclear) diluted to approx. 124 nCiml⁻¹ in BSA-TPBS. Filters were again washed three times in TPBS and positive plaques were detected by exposure of the dried filters to autoradiography film overnight at room temperature. Positive clones were picked and plaque-purified. pBluescript-SK- plasmids

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containing the chlamydial genes of interest were recovered from the purified bacteriophage using ExAssist filamentous bacteriophages (Stratagene).

Identification of antigens recognized by convalescent antisera. Recombinant plaques were identified that showed reactivity with convalescent (anti-RB) antisera, but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent antisera but not with the EB serum.

DNA Cloning and fusion protein production. The plasmid pJC2 contains a 5.0 kb *Eco*RI GPIC genomic fragment cloned into the pZEro2.1 vector (Invitrogen). To construct pJC2, the *incC* ORF sequence was ³²P-radiolabeled using random priming (Gibco-BRL) and used to probe *Eco*RI cut GPIC genomic DNA fragments separated by agarose gel electrophoresis. Fragments in the size range of the positive signal were excised from the gel and purified by Gene-Clean (Bio101). The gel-purified fragments were used in a ligation along with *Eco*RI-digested pZEro2.1. Kanamycin resistant colonies were screened by colony hybridization with radiolabeled *incC*.

MBP fusions of the five ORFs present in pJC2 were produced using the pMAL-C2 vector (New England Biolabs). The reading frame of *incC*, with the exception of the first four codons, was amplified using *Pwo* polymerase (Boehringer Mannheim) and pBS200-7 as the template. The upstream and downstream oligonucleotides for this amplification were

5'-AGAACCGATTTAACTCCAGGCG-3' (SEQ ID NO: 19) and

5'-GCGCGGATCCTTAATGTCCGGTAGGCCTAG-3' (SEQ ID NO: 20), respectively. The vector was digested with *Xmn*I and *Bam*HI, and the amplication product was digested with *Bam*HI. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incC* reading frame from pBS200-7. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E. coli* strain HD5\overline{\Overline{O}}. The resulting fusion protein (MBP/IncC) was overexpressed and purified by maltose affinity chromatography using an amylose resin supplied by New England Biolabs.

The same approach was used for production of the MBP/IncB fusion protein. The sequence encoding the N-terminal 101 amino acids of the IncB ORF was PCR amplified using the oligonucleotides

5'-ATGTCAACAACACCAGCATCTTC-3' (SEQ ID NO: 21) and 5'-GCGCGGATCCTTAATTAGTGCCTTCTGGATTAGG-3' (SEQ ID NO: 22).

The purified MBP/IncB and MBP/IncC fusion proteins were used as antigen for the production of monospecific antibody in Hartley strain guinea-pigs by standard methods (Rockey et al., 1995). Inserts in each construct were confirmed by DNA sequencing.

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For IncA, a maltose-binding protein/IncA fusion protein was produced using the pMAL-C2 vector system from New England Biolabs. The reading frame of *incA* shown in Fig.1, with the exception of the initiator ATG, the *inc*A ORF was amplified using Vent DNA polymerase (New England Biolabs) and plasmid pGP17 as template. The upstream and downstream oligonucleotides for this amplification were

5'-CGCAGTACTGTATCCACAGACAAC-3' (SEQ ID NO: 23) and

5'-GTCGGATCCGAGAAACTCTCCATGCC-3' (SEQ ID NO: 24), respectively. The vector was digested with *Xmn*l and *BamH*l, and the amplification product was digested with *Scal* and *BamH*l. Ligation of these products resulted in an in-frame fusion between the *malE* gene in the vector and the *incA* reading frame from pGP17. The stop codon for this construction is provided by the insert. Following ligation, the products were transformed into *E. coli* strain DH50. The resulting fusion protein (MBP/IncA) was overexpressed and purified by maltose affinity chromatography using amylose resin (New England Biolabs).

MBP/IncA was used as antigen for the production of mono-specific antibody reagents in Hartley strain guinea-pigs.

DNA sequencing and sequence analysis. The pBS200-7 and pJC2 genomic clones as well as the MBP fusions were sequenced with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer/Applied Biosystems Division). Several internal primers were designed to sequence further into the cloned inserts. Sequence assembly was performed using AssemblyLIGN software and sequence analysis was performed with MacVector software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Deduced amino acid sequences were compared with the database using the BLAST program (on default settings) available from the National Center for Biotechnology Information on the world wide web. The entire nucleotide sequence of the pJC2 insert was deposited in the GenBank/EMBL Nucleotide Sequence Data Library, under accession number AF017105.

For *incA*, nucleotide sequencing was conducted using the Sequences system (US Biochemical) with the M13 forward and reverse primers, and internal primers synthesized on an Milligen/Biosearch Cyclone Plus DNA synthesizer. Computer analyses were conducted using the MacVector Sequence Analysis Software (International Biotechnologies Incorporated). Hydrophilicity profiles were determined using the Kyte-Doolittle scale (Kyte and Doolittle, 1982) with a window of 7. Secondary-structure predictions were generated using a combination of the Chou-Fasman and Robson-Garnier methods (Robson and Suzuki, 1976; Chou and Fasman, 1978). Deduced amino acid sequences were compared with those in the EMBL and GenBank databases using the BLASTP program available from the National Center for Biotechnology Information.

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Electrophoresis and immunoblotting. Polyacrylamide gel electrophoresis (PAGE) was conducted using standard methods (Rockey and Rosquist, 1994). Immunoblotting was performed using standard methods (Rockey et al., 1995).

Immunofluorescence studies. Chlamydiae grown in HeLa cells on sterile glass coverslips were fixed for microscopy one of two ways. Cells were either incubated in methanol for 5 minutes, or in the combination fixative periodate-lysine-paraformaldephyde (PLP) for three hours at room temperature followed by permeabilization with 0.05% saponin (Brown and Farquhar, 1989). Immunostaining of the fixed coverslips was performed according to standard methods (Rockey et al., 1995) and visualized under a Nikon Microphot FXA microscope using the 63x objective and oil immersion.

RT-PCR analysis. RNA for RT-PCR analysis was extracted from approximately 2 x 10¹⁴ *C. psittaci*-infected cells. A Qiagen column was used for extraction and purification according to the manufacturer's instructions (Qiagen). RQ1 RNase DNase (Promega) was used to ensure removal of contaminating genomic DNA. cDNA was prepared by incubating 1.5 µg of RNA, 2.5 µM of the reverse oligonucleotide primer, and AMV reverse transcriptase (Promega) for 1 hour at 42°C in sodium pyrophosphate buffer, according to the manufacturer's instructions. PCR reactions were carried out using 1 µl of the cDNA reaction, 1.25 µM of each oligonucleotide primer, and *Pwo* polymerase (Boehringer Mannheim). Each RT-PCR reaction was accompanied by a positive control reaction that utilized the same primer set and 10 ng of *C. psittaci* genomic DNA, and a negative control reaction in which 1 µl of the same RNA preparation was used as template in the PCR reaction. A control primer set located within the *incC* gene was also used as an RT-PCR control.

Identification of incA, incB and incC genes of C. trachomatis. The nucleotide sequence information obtained for the incA, incB and incC of C. psittaci (above) was used, with standard methods, to identify the inc gene orthologues of C. trachomatis. Probes were made that corresponded to the 3' and 5' ends of the C. psittaci inc open reading frames. Standard PCR amplification (as above) was used, with the C. trachomatis genome as a template, to amplify the corresponding C. trachomatis nucleotide sequence. The amplified DNA was then sequenced, using standard methods.

2. ISOLATION OF p242, TroA AND TroB

Bacterial strains. C. trachomatis LGV-434, serotype L2, was cultivated in HeLa 229 cells using standard methods (Caldwell et al., 1981). Purified chlamydiae were obtained using Renografin (E. R. Squibb & Sons, Inc., Princton, N.J.) density gradient centrifugation (Hackstadt et al., 1992). Escherichia coli DH5II (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was used as the host strain for transformations with recombinant DNA. E. coli XL1-Blue MRF' (Stratagene, La Jolla, Calif.) was used as the host strain for infection with lambda ZAPII phage

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vector. E. coli SOLR (Stratagene) was used as the host strain for infection with in vivo excised filamentous lambda ZAPII.

Antisera. Two Cynomolgus monkeys (*Macaca fasicularis*) were anaesthetized and infected urethrally with *C. trachomatis* EBs. Each monkey was infected twice and allowed to recover between infections. Symptoms of infection were monitored over time. Antisera from infected monkeys were tested for reactivity to *Chlamydia* by ELISA (Su et al., 1990).

Sera were collected every two weeks and anti-chlamydial titers were determined. These animals showed mild clinical signs of disease which cleared spontaneously. A second challenge was then administered. Sera were collected from these animals and used to probe a *C. trachomatis* expression library as discussed below. As a control, Guinea Pigs were immunized with killed *C. trachomatis* of the EB form. Sera from these animals were obtained and also used to probe the *C. trachomatis* expression library.

C. trachomatis library construction and immunoscreening. A C. trachomatis genomic library was constructed with the lambda ZAPII vector as described above for C. psittaci. Approximately 15,000 plaques were plated, transferred to nitrocellulose filters (Schleicher and Schuell, Keene, N.H.) in duplicate, and probed with the monkey convalescent antiserum and with Guinea Pig serum against killed EBs (Bannantine et al., 1998). Plaques that reacted only with the monkey convalescent antisera were selected for further study.

Identification of antigens recognized by convalescent antisera. Four positive recombinant plaques were identified that showed reactivity with convalescent antisera but not with anti-EB serum. The purified recombinant phage were converted into pBluescriptII SK plasmid by *in vivo* excision and recircularization and these recombinant DNAs (pCt1, pCt2, pCt3 and pCt4) were used to transform *E. coli*. SDS-PAGE and immunoblot analysis of lysates of these recombinant *E. coli* showed that each expressed one or more proteins that reacted with convalescent (anti-RB) antisera but not with the anti-EB antiserum. Two of the recombinants clones, pCt2 and pCt3, expressed an identical 19.9 kDa protein (p242). The pCt4 recombinant expressed two different proteins of approximately 32 kDa each that are strongly recognized by convalescent antisera (TroA and TroB).

C. SEQUENCE ANALYSIS

Sequence analysis of pCt1, 2, and 3 revealed overlapping inserts with only one open reading frame (ORF) common in all three. This ORF encodes an approximately 19.9 kDa protein (p242) that shows no similarity to other known proteins. The nucleotide sequence encoding *C. trachomatis* p242, and the amino acid sequence of the protein are shown in SEQ ID NOS:1 and 2, respectively.

The insert in pCt4 contains two complete ORFs which code for two proteins, each of approximately 32kDa (TroA and TroB) that show some homology with proteins from *Treponema*

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pallidum. The nucleotide sequences encoding the 32 kDa proteins (TroA and TroB) and the amino acid sequences of these proteins are shown in SEQ ID NOS: 3, 4, 5, and 6.

D. EMBODIMENTS OF THE INVENTION

The present invention includes the nucleotide and amino acid sequences for certain infection-specific proteins from *Chlamydia*. These proteins are p242, TroA, and TroB from *C. trachomatis*, and the IncB, and IncC proteins from *C. psittaci*. The scope of the invention includes fragments of these proteins that may be used in a vaccine preparation or that may be used in a method of detecting *Chlamydia* antibodies. Such fragments may be, for example, 5, 10, 15, 20, 25, or 30 contiguous amino acids in length, or may even encompass the entire protein.

The present invention also encompasses the use of infection-specific proteins of *Chlamydia*, and the use of nucleotides encoding such proteins. Infection-specific proteins include the IncA, IncB and IncC proteins of *C. psittaci*, the IncA, IncB and IncC proteins of *C. trachomatis*, and the TroA, TroB, and p242 proteins of *C. trachomatis*. The inventors have shown that these proteins are infection-specific by using immunological techniques such as immunofluorescence microscopy and immunoblotting.

The present invention includes a vaccine against chlamydial infections comprising infection-specific proteins or fragments of these proteins or proteins that are homologous or show substantial sequence similarity to these proteins. In one embodiment, one or more purified infection-specific proteins may be mixed with a pharmaceutically acceptable excipient to produce a vaccine that stimulates a protective immunological response in an animal. In one embodiment the vaccine may be administered intra-muscularly or sub-cutaneously or intravenously. In another embodiment, the vaccine may be administered by inoculation into or onto the mucous membranes of the subject animal. For example, the vaccine may be administered urethrally or genitally as a liquid or in the form of a pessary. In another embodiment, it may be administered to the mucosa of the lungs as a spray or vapor suspension.

Since at least three amino acids are required to produce an antigenic epitope, the vaccine should comprise at least three consecutive amino acids, preferably at least five consecutive amino acids, and may comprise at least 10, 15, 25, 30, 40, or 45 consecutive amino acids of the infection-specific proteins as shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, and 18.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by C. psittaci, C. trachomatis, C. pneumoniae or C. pecorum. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific Chlamydia antigen.

Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response (e.g., alum). The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether

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through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*. Protective antibodies may be elicited by a series of two or three doses of the antigenic vaccine given about two weeks apart.

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The present invention also teaches a method of making a vaccine against chlamydial infections. The method of making the vaccine comprises providing a pure (or substantially pure) infection-specific chlamydial peptide or portion thereof, and mixing the peptide with a pharmacologically acceptable excipient or adjuvant. Adjuvants may include commonly used compounds such as alum. Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine and characteristics of the animal or human patient to be vaccinated.

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The infection-specific vaccine of the invention is directed towards not only *C. psittaci*, but against all forms of *Chlamydia* including *C. pneumoniae*, *C. trachomatis* and *C. pecorum*, and the vaccine may comprise not just peptides derived from *C. psittaci*, but also orthologous peptides and fragments of such orthologous peptides from other species of *Chlamydia* and peptides that are substantially similar to such peptides.

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The present invention also teaches a method of vaccination comprising administering a vaccine formulated as described above to an animal either intravenously, intramuscularly, subcutaneously, by inhalation of a vapor or mist, or by inoculation in the form of a liquid, spray, ointment, pessary or pill into or onto the mucous membranes of the mouth, nose, lungs or urogenital tract or colon.

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The methods of the invention may be practiced equally with human or non-human animal subjects.

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The present invention also teaches a method of detecting *Chlamydia* infection-specific proteins produced by the Reticulate Body form of the organism. In this embodiment, antibodies raised to the infection-specific proteins are used in an immunological assay such as an Enzyme Linked Immunosorbant Assay or Biotin-Avidin assay or a radioimmunoassay or any other assay wherein specific antibodies are used to recognize a specific protein. Such assays may be used to detect both the quantity of proteins present and also the specificity of binding of such proteins. In such an assay, antibodies have attached to them, usually at the *Fc* portion, a detectable label, such as an enzyme, fluorescent marker, a radioactive marker or a Biotin-Avidin system marker that allows detection. A biological sample is provided from an animal that has been putatively exposed to *Chlamydia*. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. The sample is then contacted with the labeled antibody and specific binding, if any, is detected. Other methods of using infection-specific antibodies to detect infection-specific

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antigens that are present in cells or tissues include immunofluorescense, indirect-immunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an anti-immunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of flourescent emissions from the dye moiety. This technique would be particularly useful, for instance, for detection of *Chlamydia* antigen present on a urogenital mucosal smear.

Other techniques, such as competitive inhibition assays may also be used to assay for antigen, and one of ordinary skill in the art will readily appreciate that the precise methods disclosed may be modified or varied without departing from the subject or spirit of the invention taught herein.

The present invention also teaches a method of detection of *Chlamydia* infection-specific antibodies made against the Reticulate Body. In this embodiment a sample is provided from an animal putatively exposed to *Chlamydia* to determine whether the sample contains infection-specific antibodies. Such a sample may be, for example, whole blood, serum, tissue, saliva or a mucosal secretion. This sample is contacted with infection-specific antigens such that the amount and specificity of binding of the antibody may be measured by its binding to a specific antigen. Many techniques are commonly known in the art for the detection and quantification of antigen. Most commonly, the purified antigen will be bound to a substrate, the antibody of the sample will bind via its *Fab* portion to this antigen, the substrate will then be washed and a second, labeled antibody will then be added which will bind to the *Fc* portion of the antibody that is the subject of the assay. The second, labeled antibody will be species specific, i.e., if the serum is from a human, the second, labeled antibody will be anti-human-IgG antibody. The specimen will then be washed and the amount of the second, labeled antibody that has been bound will be detected and quantified by standard methods.

The present invention also teaches a method of treating a *Chlamydial* infection by directing a therapeutic agent against a specific target, such as: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

For example, the present invention teaches a method of treating chlamydial infection wherein antisense technology is used to prevent the expression of infection-specific genes, thereby preventing the pathologies associated these proteins and preventing reproduction of the RB phase of *Chlamydia*. In this embodiment, RNA molecules complementary to transcripts of infection specific genes are introduced into the host cells that contain *Chlamydia*, and by binding to the mRNA transcripts of the infection-specific genes, prevent translation and therefore expression of the infection-specific proteins that are associated with pathogenesis.

The invention may be practiced to produce a vaccine against any species of *Chlamydia*, including *C. psittaci*, *C. pecorum*, *C. trachomatis and C. pneumoniae*.

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The following examples illustrate various embodiments of the invention.

EXAMPLE 1: Homologous Sequences

The DNA and protein sequences discussed herein are shown in SEQ ID NOS:1-18. These sequences refer to infection-specific proteins and to the DNA sequences that encode these proteins. Although these sequences are from *C. psittaci* and *C. trachomatis*, it would be equally possible to substitute in the present invention, the orthologs of these sequences from other *Chlamydia* species such as *C. pecorum* and *C. pneumoniae*.

Such orthologous sequences may be obtained from the appropriate organisms by isolation of the genome of the organism, digestion with restriction enzymes, separation of restriction fragments by electrophoresis and purification of these fragments and selection of fragments of appropriate size. Identity of the fragments can be confirmed by dot-blot and by standard DNA sequencing techniques. The orthologous sequences in different *Chlamydia* species may also be found by selection of appropriate PCR primers (selected from appropriate regions flanking the *Chlamydia* gene of interest), and the use of these primers in a PCR reaction, using the genome of the particular species of *Chlamydia* of interest as a template, to amplify the ortholog of interest. Such PCR primers would be selected from the flanking regions to allow specific amplification of the target gene. The fragments so obtained could then be run on a gel to check size and sequenced and compared against the known sequences to determine sequence identity.

The degree of sequence identity between the infection-specific genes of *C. psittaci* or *C. trachomatis* and their orthologs from *C. pecorum* and *C. pneumoniae*, may be determined by comparing sequences using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) as described herein.

Orthologues of interest infection-specific proteins are characterized by possession of at least 50% or greater sequence identity counted over the full length alignment with one of the disclosed amino acid sequences of the *C. psittaci* or *C. trachomatis* infection-specific proteins using gapped blastp set to default parameters (described herein).

EXAMPLE 2: Heterologous Expression of Infection-Specific Antigens

Methods for expressing large amounts of protein from a cloned gene introduced into Escherichia coli (E. coli) may be utilized for the purification of the Chlamydia peptides. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are well known and are described in Sambrook et al. (1989). Such fusion proteins may be made in large amounts, are relatively simple to purify, and can be used to produce antibodies. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome binding site upstream of the cloned gene. If low levels of protein are produced, additional steps

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may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy.

Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described in chapter 17 of Sambrook et al. (1989). Vector systems suitable for the expression of *lacZ* fusion genes include the pUC series of vectors (Ruther et al. (1983)), pEX1-3 (Stanley and Luzio (1984)) and pMR100 (Gray et al. (1982)). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg (1981)), pKK177-3 (Amann and Brosius (1985)) and pET-3 (Studiar and Moffatt (1986)).

Fusion proteins may be isolated from protein gels, lyophilized, ground into a powder and used as antigen preparations.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, amphibian or avian species, may also be used for protein expression, as is well known in the art. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other prokaryotic and eukaryotic cells and cell lines may be appropriate for a variety of purposes, e.g., to provide higher expression, post-translational modification, desirable glycosylation patterns, or other features.

Additionally, peptides, particularly shorter peptides, may be chemically synthesized, avoiding the need for purification from cells or culture media. It is known that peptides as short as 3 amino acids can act as an antigenic determinant and stimulate an immune response. Such peptides may be administered as vaccines in ISCOMs (Immune Stimulatory Complexes) as described by Janeway & Travers, Immunobiology: The Immune System In Health and Disease, 13.21 (Garland Publishing, Inc. New York, 1997). Accordingly, one aspect of the present invention includes small peptides encoded by the nucleic acid molecules disclosed herein. Such peptides include at least 5, and may be at least 10, 15, 20, 25, or 30 or more contiguous amino acids of the polypeptide sequences described herein.

EXAMPLE 3: Production of Antibodies Specific for Infection-Specific Antigens

Antibody against infection-specific antigen is encompassed by the present invention, particularly for the detection of *Chlamydia* infection-specific antigen. Such antibody may be produced by inoculation of an animal such as a guinea-pig or a monkey with infection-specific antigen produced as described above. Such antigen may be a polypeptide as disclosed herein, such as a complete or partial polypeptide from *C. psittaci*, *C. trachomatis*, *C. pneumoniae* or *C. pecorum*. As discussed above, any molecule that can elicit a specific, protective immune response

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may be used as a vaccine, but since a minimum of three amino acids are required to do this, a vaccine should comprise at least three amino acids.

The peptide for use in the vaccine of the invention may be naturally derived or may be synthetic such as those synthesized on a commercially available peptide synthesizer. The peptide may also comprise a complete or partial peptide derived from the *C. pneumoniae* or *C. pecorum* infection-specific orthologs of the *C. trachomatis* or *C. psittaci* proteins as set out herein.

In one method of production, a polyclonal antibody is produced by providing a purified peptide which is diluted to 100 micrograms per milliliter in sterile saline and mixed with RiBi Trivalent Adjuvant (RiBi Immunochem Inc). The antigen/adjuvant emulsion is then administered to an anaesthetized guinea pig using a procedure as provided by the manufacturer. Serum is collected 14 days after secondary and tertiary immunizations.

Monoclonal antibody to epitopes of the *Chlamydia* peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected purified protein over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin, e.g., Hypoxanthene, Aminopterin and Thymidine (HAT) medium. The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane (1988).

An alternative approach to raising antibodies against the *Chlamydia* peptides is to use synthetic peptides synthesized on a commercially available peptide synthesizer based upon the amino acid sequence of the peptides predicted from nucleotide sequence data.

In another embodiment of the present invention, monoclonal antibodies that recognize a specific *Chlamydia* peptide are produced. Optimally, monoclonal antibodies will be specific to each peptide, i.e., such antibodies recognize and bind one *Chlamydia* peptide and do not substantially recognize or bind to other proteins, including those found in uninfected human cells.

The determination that an antibody specifically detects a particular *Chlamydia* peptide is made by any one of a number of standard immunoassay methods; for instance, the western blotting technique (Sambrook et al., 1989). To determine that a given antibody preparation (for instance from a guinea pig) specifically detects one *Chlamydia* peptide by western blotting, total cellular protein is extracted from a sample of blood from an unexposed subject and from a sample of blood from an exposed subject. As a positive control, total cellular protein is also extracted from

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Chlamydia cells grown in vitro. These protein preparations are then electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. Thereafter, the proteins are transferred to a membrane (for example, nitrocellulose) by western blotting, and the antibody preparation is incubated with the membrane. After washing the membrane to remove non-specifically bound antibodies, the presence of specifically bound antibodies is detected by the use of an anti-guinea pig antibody conjugated to an enzyme such as alkaline phosphatase; application of the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium results in the production of a dense blue compound by immuno-localized alkaline phosphatase. Antibodies which specifically detect the Chlamydia protein will, by this technique, be shown to bind to the Chlamydia-extracted sample at a particular protein band (which will be localized at a given position on the gel determined by its molecular weight) and to the proteins extracted from the blood of the exposed subject. No significant binding will be detected to proteins from the unexposed subject.

EXAMPLE 4: Use of Infection-Specific Sequences and their Corresponding Peptides and Antibodies in Diagnostic Assays

Another aspect of the present invention is a method for detecting the presence of anti-Chlamydia antibodies that react with infection-specific Chlamydia proteins, Chlamydia peptides and Chlamydia nucleic acid sequences in biological samples. These methods include detection of antigen and antibody by ELISA and similar techniques, the detection of proteins in a tissue sample by immunofluorescence and related techniques and the detection of specific DNA sequences by specific hybridization and amplification.

One aspect of the invention is an ELISA that detects anti-Chlamydia antibodies in a medical specimen. An immunostimulatory infection-specific Chlamydia peptide of the present invention is employed as an antigen and is preferably bound to a solid matrix such as a crosslinked dextran such as SEPHADEX (Pharmacia, Piscataway, NJ), agarose, polystyrene, or the wells of a microtiter plate. The polypeptide is admixed with the specimen, such as blood, and the admixture is incubated for a sufficient time to allow antibodies present in the sample to immunoreact with the polypeptide. The presence of the positive immunoreaction is then determined using an ELISA assay, usually involving the use of an enzyme linked to an anti-immunoglobulin that catalyzes the conversion of a chromogenic substrate.

In one embodiment, the solid support to which the polypeptide is attached is the wall of a microtiter assay plate. After attachment of the polypeptide, any nonspecific binding sites on the microtiter well walls are blocked with a protein such as bovine serum albumin. Excess bovine serum albumin is removed by rinsing and the medical specimen is admixed with the polypeptide in the microtiter wells. After a sufficient incubation time, the microtiter wells are rinsed to remove excess sample and then a solution of a second antibody, capable of detecting human antibodies is added to the wells. This second antibody is typically linked to an enzyme such as peroxidase,

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alkaline phosphatase or glucose oxidase. For example, the second antibody may be a peroxidase-labeled goat anti-human antibody. After further incubation, excess amounts of the second antibody are removed by rinsing and a solution containing a substrate for the enzyme label (such as hydrogen peroxide for the peroxidase enzyme) and a color-forming dye precursor, such as o-phenylenediamine is added. The combination of *Chlamydia* peptide (bound to the wall of the well), the human anti-*Chlamydia* antibodies (from the specimen), the enzyme-conjugated anti-human antibody and the color substrate will produce a color that can be read using an instrument that determines optical density, such as a spectrophotometer. These readings can be compared to a negative control such as a sample known to be free of anti-*Chlamydia* antibodies. Positive readings indicate the presence of anti-*Chlamydia* antibodies in the specimen, which in turn indicate a prior exposure of the patient to *Chlamydia*.

In another embodiment, antibodies that specifically recognize a Chlamydia peptide encoded by the nucleotide sequences disclosed herein are useful in diagnosing the presence of infection-specific Chlamydia antigens in a subject or sample. For example, detection of infectionspecific antigens that are present in cells or tissues may be done by immunofluorescence, indirectimmunofluorescense and immunohistochemistry. In immunofluorescense, a fluorescent dye is bound directly to the antibody. In indirect-immunofluorescence, the dye is bound to an antiimmunoglobulin. Specific binding occurs between antigen and bound antibody is detected by virtue of fluorescent emissions from the dye moiety. This technique may be particularly useful. for instance, for detection of Chlamydia antigen present on a urogenital mucosal smear. Chlamydia may be present in urogenital mucosa, and a smear on a glass slide may be fixed and bathed in a solution containing an antibody specific to the infection-specific antigen. The slide is then washed to remove the unbound antibody, and a fluorescent anti-immunoglobulin antibody is added. The slide is washed again, and viewed microscopically under an appropriate wavelength of light to detect fluorescence. Fluorescence indicates the presence of Chlamydia antigen. Alternatively, a urogenital mucosal smear may be taken, the sample cultured with HeLa cells to produce large amounts of the RB form, and immunofluorescence may then be used to detect infection-specific Chlamydia antibodies.

Another aspect of the invention includes the use of nucleic acid primers to detect the presence of *Chlamydia* nucleic acids that encode infection-specific antigens in body samples and thus to diagnose infection. In other embodiments, these oligonucleotide primers will comprise at least 15 contiguous nucleotides of a DNA sequence as shown in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, or 17. In other embodiments, such oligonucleotides may comprise at least 20 or at least 25 or more contiguous nucleotides of the aforementioned sequences.

One skilled in the art will appreciate that PCR primers are not required to exactly match the target gene sequence to which they anneal. Therefore, in another embodiment, the oligonucleotides will comprise a sequence of at least 15 nucleotides and preferably at least 20 nucleotides, the oligonucleotide sequence being substantially similar to a DNA sequence set forth

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in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, and 17. Such oligonucleotides may share at least about 75%, 85%, 90% or greater sequence identity.

The detection of specific nucleic acid sequences in a sample by polymerase chain reaction amplification (PCR) is discussed in detail in Innis et al., (1990). PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, part 4 in particular. To detect Chlamydia sequences, primers based on the sequences disclosed herein would be synthesized, such that PCR amplification of a sample containing Chlamydia DNA would result in an amplified fragment of a predicted size. If necessary, the presence of this fragment following amplification of the sample nucleic acid could be detected by dot blot analysis. PCR amplification employing primers based on the sequences disclosed herein may also be employed to quantify the amounts of Chlamydia nucleic acid present in a particular sample (see chapters 8 and 9 of Innis et al., (1990)).

Alternatively, probes based on the nucleic acid sequences described herein may be labeled with suitable labels (such a P³² or biotin) and used in hybridization assays to detect the presence of *Chlamydia* nucleic acid in provided samples.

Reverse-transcription PCR using these primers may also be utilized to detect the presence of *Chlamydia* RNA which is indicative of an ongoing infection.

EXAMPLE 5: Production of Chlamydia Vaccines

The purified peptides of the present invention may be used directly as immunogens for vaccination. Methods for using purified peptides as vaccines are well known in the art and are described in Yang et al. (1991), Andersen (1994) and Jardim et al. (1990). As is well known in the art, adjuvants such as alum, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used in formulations of purified peptides as vaccines. Accordingly, one embodiment of the present invention is a vaccine comprising one or more immunostimulatory *C. trachomatis* or *C. psittaci* peptides encoded by nucleotide sequences as shown in the attached sequence listing, together with a pharmaceutically acceptable adjuvant.

Additionally a vaccine may comprise a defined fraction of the disclosed peptide of *C. trachomatis* or *C. psittaci* or may comprise a peptide wherein the gene coding for the peptide shows substantial similarity to the DNA sequences disclosed herein, such as for orthologous genes of *C. pneumoniae* or *C. pecorum*.

Additionally, the vaccines may be formulated using a peptide according to the present invention together with a pharmaceutically acceptable excipient such as water, saline, dextrose and glycerol. The vaccines may also include auxiliary substances such as emulsifying agents and pH buffers.

It will be appreciated by one of skill in the art that vaccines formulated as described above may be administered in a number of ways including subcutaneous, intra-muscular and intra-venous injection. Doses of the vaccine administered will vary depending on the antigenicity of the particular peptide or peptide combination employed in the vaccine, and characteristics of the

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animal or human patient to be vaccinated. While the determination of individual doses will be within the skill of the administering physician, it is anticipated that doses of between 1 microgram and 1 milligram will be employed.

As with many vaccines, the vaccines of the present invention may routinely be administered several times over the course of a number of weeks to ensure that an effective immune response is triggered. Where such multiple doses are administered, they will normally be administered at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, may be desirable to maintain the desired levels of protective immunity.

Alternatively, multiple immunostimulatory peptides may also be administered by expressing the nucleic acids encoding the peptides in a nonpathogenic microorganism, and using this transformed nonpathogenic microorganism as a vaccine.

Finally, a recent development in the field of vaccines is the direct injection of nucleic acid molecules encoding peptide antigens, as described in Janeway & Travers, (1997). Thus, plasmids which include nucleic acid molecules described herein, or which include nucleic acid sequences encoding peptides according to the present invention may be utilized in such DNA vaccination methods.

The vaccine of the invention may be used to inoculate potential animal targets of any of the chlamydial diseases including those caused by *C. trachomatis*, *C. psittaci*, *C. pneumoniae* or *C. pecorum*. Indeed the vaccine of the invention may be used to inoculate animals against any disease that shows immunological cross-protection as a result of exposure to infection-specific *Chlamydia* antigen. The protein or polypeptide is present in the vaccine in an amount sufficient to induce a protective immune response whether through humoral or cell mediated pathways or through both. Such a response protects the immunized animal against chlamydial infections specifically by raising an immune response against the Reticulate Body form of *Chlamydia*.

The above embodiments are set out only by way of example and are not intended to be exclusive, one skilled in the art will understand that the invention may be practiced in various additional ways without departing from the subject of the spirit of the invention.

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CLAIMS

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What is claimed is:

1. A purified infection-specific protein comprising an amino acid sequence selected from the group consisting of:

5 (a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 10,

(e) SEQ ID NO: 12,

(f) an amino acid sequence that differs from an amino acid sequence of (a) to (e) inclusive, by one or more conservative amino acid substitutions, and

(g) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (e) inclusive.

- 2. An isolated nucleic acid molecule encoding a protein according to claim 1.
- 3. An isolated nucleic acid molecule according to claim 2 wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) SEQ ID NO: 1,

(b) SEQ ID NO: 3,

(c) SEQ ID NO: 5,

(d) SEQ ID NO: 9, and

(e) SEQ ID NO: 11.

- 4. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleotide molecule according to claim 2.
- 5. A vaccine preparation comprising at least one purified peptide comprising at least 5 contiguous amino acids selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

30 (e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

- 6. The vaccine preparation of claim 5 wherein the peptide comprises at least 10 contiguous amino acids of at least one of the specified sequences.
 - 7. The vaccine preparation of claim 5 wherein the peptide comprises at least 15 contiguous amino acids of at least one of the specified sequences.

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- 8. The vaccine preparation of claim 5 wherein the purified peptide comprises at least 20 contiguous amino acids of at least one of the specified sequences.
- 9. A vaccine preparation comprising an amino acid sequence selected from the5 group consisting of:
 - (a) SEQ ID NO: 2,
 - (b) SEQ ID NO: 4,
 - (c) SEQ ID NO: 6,
 - (d) SEQ ID NO: 8,
- 10 (e) SEQ ID NO: 10,
 - (f) SEQ ID NO: 12,
 - (g) SEQ ID NO: 14,
 - (h) SEQ ID NO: 16,
 - (i) SEQ ID NO: 18,
 - (j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions, and
 - (k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive.
- A method of making a vaccine comprising combining a pharmaceutically
 acceptable excipient with a purified peptide having an amino acid sequence selected from the group consisting of:
 - (a) SEQ ID NO:2,
 - (b) SEQ ID NO:4,
 - (c) SEQ ID NO:6,
- 25 (d) SEQ ID NO:8,
 - (e) SEQ ID NO:10,
 - (f) SEQ ID NO:12,
 - (g) SEQ ID NO:14,
 - (h) SEQ ID NO:16,
- 30 (i) SEQ ID NO:18,
 - (j) an amino acid sequence that differs from an amino acid sequence of (a) to (i) inclusive, by one or more conservative amino acid substitutions,
 - (k) an amino acid sequence having at least 60% sequence identity to an amino acid sequence of (a) to (i) inclusive, and
- 35 (l) at least 10 contiguous amino acids from an amino acid sequence of (a) to (i) inclusive.
 - 11. A method of vaccination, comprising administering a vaccine preparation according to claim 5 to a mammal.

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- 12. A method of vaccination, comprising administering a vaccine preparation according to claim 9 to a mammal.
- 13. A method of detecting an infection-specific *Chlamydia* protein in a biological sample comprising: contacting the biological sample with at least one anti-*Chlamydia* antibody, which antibody is an infection-specific antibody, such that a reaction between the antibody and the infection-specific *Chlamydia* protein gives rise to a detectable effect, and detecting the detectable effect.
- 14. The method of claim 13 wherein the anti-*Chlamydia* antibody binds specifically to a peptide having an amino acid sequence selected from the group consisting of:

10 (a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

15 (f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

- 15. A method of detecting an infection-specific anti-Chlamydia antibody in a biological sample comprising: contacting the biological sample with at least one Chlamydia peptide, which peptide is an infection specific peptide, such that a reaction between the peptide and the infection-specific anti-Chlamydia antibody gives rise to a detectable effect, and detecting the detectable effect.
- 16. The method of claim 15 wherein the *Chlamydia* peptide comprises at least 5 contiguous amino acids of a sequence selected from the group consisting of:

(a) SEQ ID NO: 2,

(b) SEQ ID NO: 4,

(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

- 17. The method of claim 15 wherein said *Chlamydia* peptide comprises an amino acid sequence selected from the group consisting of:
 - (a) SEQ ID NO: 2,
 - (b) SEQ ID NO: 4,

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(c) SEQ ID NO: 6,

(d) SEQ ID NO: 8,

(e) SEQ ID NO: 10,

(f) SEQ ID NO: 12,

(g) SEQ ID NO: 14,

(h) SEQ ID NO: 16, and

(i) SEQ ID NO: 18.

18. A method of treating a *Chlamydial* infection comprising directing a therapeutic agent against a specific target, said target chosen from the group consisting of: (i) an infection-specific protein of *Chlamydia*, (ii) a gene that encodes an infection-specific protein of *Chlamydia* and (iii) an RNA transcript that encodes an infection-specific protein of *Chlamydia*, wherein said therapeutic agent interacts with said target to affect a reduction in pathology.

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SEQUENCE LISTING

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Gly Leu Ser Glu Thr Ala Ala Ala Glu Leu Arg Lys Lys Phe Glu Asp

Leu Ser Ala Glu Tyr Asn Thr Ala Gln Gly Gln Tyr Tyr Gln Ile Leu 105

Asn Gln Ser Asn Phe Lys Arg Met Gln Lys Ile Met Glu Glu Val Lys 115 120 125

Lys Ala Ser Glu Thr Val Arg Ile Gln Glu Gly Leu Ser Val Leu Leu 135

Asn Glu Asp Ile Val Leu Ser Ile Asp Ser Ser Ala Asp Lys Thr Asp 145 150 155

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Ser

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WO 99/53948 PCT/US99/08744 ggg ttg tct cct gag gct cag att agt atc cga gat att atg cgt gta Gly Leu Ser Pro Glu Ala Gln Ile Ser Ile Arg Asp Ile Met Arg Val 195 200 gtg gag tat atc tct gca aac gat gta gaa gtt gtc ttt tta gag gat 672 Val Glu Tyr Ile Ser Ala Asn Asp Val Glu Val Val Phe Leu Glu Asp 215 acg tta aat caa gat gct ttg aga aag att gtt tct tgc tct aag agc 720 Thr Leu Asn Gln Asp Ala Leu Arg Lys Ile Val Ser Cys Ser Lys Ser 230 gga caa aag att cgt ctc gct aag tct cct tta tat agc gat aat gtc 768 Gly Gln Lys Ile Arg Leu Ala Lys Ser Pro Leu Tyr Ser Asp Asn Val 245 250 tgt gat aac tat ttt agc acg ttc cag cac aat gtt cgc aca att aca Cys Asp Asn Tyr Phe Ser Thr Phe Gln His Asn Val Arg Thr Ile Thr 265 gaa gaa ttg gga ggg act gtt ctt gaa tag 846 Glu Glu Leu Gly Gly Thr Val Leu Glu 275 <210> 4 <211> 281 <212> PRT <213> Chlamydia trachomatis <400> 4 Met Asn Arg Met Ile Cys Asp Cys Val Ser Arg Ile Thr Gly Asp Arg Val Lys Asn Ile Val Leu Ile Asp Gly Ala Ile Asp Pro His Ser Tyr 25 Glu Met Val Lys Gly Asp Glu Asp Arg Met Ala Met Ser Gln Leu Ile Phe Cys Asn Gly Leu Gly Leu Glu His Ser Ala Ser Leu Arg Lys His Leu Glu Gly Asn Pro Lys Val Val Asp Leu Gly Gln Arg Leu Leu Asn Lys Asn Cys Phe Asp Leu Leu Ser Glu Glu Gly Phe Pro Asp Pro His Ile Trp Thr Asp Met Arg Val Trp Gly Ala Ala Val Lys Glu Met Ala 110 Ala Ala Leu Ile Gln Gln Phe Pro Gln Tyr Glu Glu Asp Phe Gln Lys Asn Ala Asp Gln Ile Leu Ser Glu Met Glu Glu Leu Asp Arg Trp Ala 130 135 Val Arg Ser Leu Ser Thr Ile Pro Glu Lys Asn Arg Tyr Leu Val Thr 145 150 155

Gly His Asn Ala Phe Ser Tyr Phe Thr Arq Arq Tyr Leu Ser Ser Asp 170 Ala Glu Arg Val Ser Gly Glu Trp Arg Ser Arg Cys Ile Ser Pro Glu 180 Gly Leu Ser Pro Glu Ala Gln Ile Ser Ile Arg Asp Ile Met Arg Val Val Glu Tyr Ile Ser Ala Asn Asp Val Glu Val Val Phe Leu Glu Asp Thr Leu Asn Gln Asp Ala Leu Arg Lys Ile Val Ser Cys Ser Lys Ser 230 235 Gly Gln Lys Ile Arg Leu Ala Lys Ser Pro Leu Tyr Ser Asp Asn Val Cys Asp Asn Tyr Phe Ser Thr Phe Gln His Asn Val Arg Thr Ile Thr 265 Glu Glu Leu Gly Gly Thr Val Leu Glu <210> 5 <211> 861 <212> DNA <213> Chlamydia trachomatis <220> <221> CDS <222> (1)..(861) <400> 5 atg tet gtg ata act att tta gca egt tee age aca atg tte gca caa Met Ser Val Ile Thr Ile Leu Ala Arg Ser Ser Thr Met Phe Ala Gln tta cag aag aat tgg gag gga ctg ttc ttg aat aga gat aat qca att Leu Gln Lys Asn Trp Glu Gly Leu Phe Leu Asn Arg Asp Asn Ala Ile 25 get tgg tee gta gag gat ett tgt gtt aat tat gat eac tea gae gte Ala Trp Ser Val Glu Asp Leu Cys Val Asn Tyr Asp His Ser Asp Val tta tgt cac att act ttt tct ctg cct gca ggg gca atg gct gct att Leu Cys His Ile Thr Phe Ser Leu Pro Ala Gly Ala Met Ala Ala Ile att ggg ccg aat gga gct ggt aaa agt act ttg ctt aag gct tct tta Ile Gly Pro Asn Gly Ala Gly Lys Ser Thr Leu Leu Lys Ala Ser Leu gga ctg att cgt gct tct tct ggc caa agc ttg ttc ttt ggt cag aga Gly Leu Ile Arg Ala Ser Ser Gly Gln Ser Leu Phe Phe Gly Gln Arg 85 90 ttt tcc aag gca cat cat aga ata gcc tat atg cct caa aga gcg agt Phe Ser Lys Ala His His Arg Ile Ala Tyr Met Pro Gln Arg Ala Ser

100 105 110

											ttg Leu			384
											gat Asp			432
											gct Ala			480
											aga Arg			528
											atg Met 190			576
											gta Val			624
											att Ile			672
											tta Leu			720
_		 			-	-	-	-	_	_	act Thr		- .	768
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<211> 286

<212> PRT

<213> Chlamydia trachomatis

<400> 6

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Ala Trp Ser Val Glu Asp Leu Cys Val Asn Tyr Asp His Ser Asp Val 35 40 45

Leu Cys His Ile Thr Phe Ser Leu Pro Ala Gly Ala Met Ala Ala Ile 50 55 60

- Ile Gly Pro Asn Gly Ala Gly Lys Ser Thr Leu Leu Lys Ala Ser Leu 65 70 75 80
- Gly Leu Ile Arg Ala Ser Ser Gly Gln Ser Leu Phe Phe Gly Gln Arg
- Phe Ser Lys Ala His His Arg Ile Ala Tyr Met Pro Gln Arg Ala Ser
- Val Asp Trp Asp Phe Pro Met Thr Val Leu Asp Leu Val Leu Met Gly 115 120 125
- Cys Tyr Gly Tyr Lys Gly Ile Trp Asn Arg Ile Ser Thr Asp Asp Arg 130 135
- Gln Glu Ala Met Arg Ile Leu Glu Arg Val Gly Leu Glu Ala Phe Ala 145 150 155 160
- Asn Arg Gln Ile Gly Lys Leu Ser Gly Gly Gln Gln Arg Ala Phe 165 170 175
- Leu Ala Arg Ser Leu Met Gln Lys Ala Asp Leu Tyr Leu Met Asp Glu 180 185 190
- Leu Phe Ser Ala Ile Asp Met Ala Ser Tyr Gln Met Val Val Asp Val 195 200 205
- Leu Gln Glu Leu Lys Ser Glu Gly Lys Thr Ile Val Val Ile His His 210 220
- Asp Leu Ser Asn Val Arg Lys Leu Phe Asp His Val Ile Leu Leu Asn 225 230 235 240
- Lys His Leu Val Cys Ser Gly Ser Val Glu Glu Cys Leu Thr Lys Glu 245 250 255
- Ala Ile Phe Gln Ala Tyr Gly Cys Asp Leu Ser Phe Trp Ile Thr His 260 265 270
- Ser Asn Cys Leu Glu Ala Ser Thr Lys Asp Arg Ala Arg Cys 275 280 285
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 Met Thr Val Ser Thr Asp Asn Thr Ser Pro Val Ile Ser Arg Ala Ser

 1 5 10 15

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tca cct act ttt gga gat cat ggt aag gat ttc gac aac aat aaa att Ser Pro Thr Phe Gly Asp His Gly Lys Asp Phe Asp Asn Asn Lys Ile ata ccc att tca ata gaa gct cca act tct tca gct gct gct gta ggg Ile Pro Ile Ser Ile Glu Ala Pro Thr Ser Ser Ala Ala Ala Val Gly gct aaa acg gct atc gag cct gaa gga aga agc cca cta ctt caa agg 192 Ala Lys Thr Ala Ile Glu Pro Glu Gly Arg Ser Pro Leu Leu Gln Arg att tgc tat ctt gtt aaa att atc gct gcc atc gcc ctc ttt gtt gtt 240 Ile Cys Tyr Leu Val Lys Ile Ile Ala Ile Ala Leu Phe Val Val ggt atc gca gcc tta gtt tgc tta tat ctc ggt agc gtt atc tca acq 288 Gly Ile Ala Ala Leu Val Cys Leu Tyr Leu Gly Ser Val Ile Ser Thr 85 cct tct ctt att ctt atg ctt gcg atc atg ctt gta tcc ttt gtg atc Pro Ser Leu Ile Leu Met Leu Ala Ile Met Leu Val Ser Phe Val Ile 100 105 gtt att acg gca att cga gat ggc aca ccg tct caa gtg gtc cgt cac Val Ile Thr Ala Ile Arg Asp Gly Thr Pro Ser Gln Val Val Arg His 115 120 atg aaa cag caa att cag caa ttt ggc gaa gaa aac acg cgt tta cat Met Lys Gln Gln Ile Gln Gln Phe Gly Glu Glu Asn Thr Arg Leu His 130 acc qca qta qaa aat cta aaa gct qtt aac gtt gag ctc tca gag caa Thr Ala Val Glu Asn Leu Lys Ala Val Asn Val Glu Leu Ser Glu Gln 145 150 att aac caa ctt aaa caa cta cat act aga tta tcg gat ttt ggt gat 528 Ile Asn Gln Leu Lys Gln Leu His Thr Arg Leu Ser Asp Phe Gly Asp 165 170 175 agg ctt gaa gcg aat acc ggt gat ttt act gca ctt att gcg gat ttc Arg Leu Glu Ala Asn Thr Gly Asp Phe Thr Ala Leu Ile Ala Asp Phe 180 caa ctc agt ctg gaa gag ttt aag tct gtt ggt act aaa gtt gaa acc 624 Gln Leu Ser Leu Glu Glu Phe Lys Ser Val Gly Thr Lys Val Glu Thr 195 atg ctc tct cca ttt gag aaa tta gct cag tct ttg aaa gag acc ttt 672 Met Leu Ser Pro Phe Glu Lys Leu Ala Gln Ser Leu Lys Glu Thr Phe 210 215 tct caa gaa gct gtt cag gca atg tcc tct gta act gag tta aga 720 Ser Gln Glu Ala Val Gln Ala Met Met Ser Ser Val Thr Glu Leu Arg 225 230 235 acc aat ttg aat gca ttg aaa gag ctt ata aca gag aat aaa acc gta 768

Thr Asn Leu Asn Ala Leu Lys Glu Leu Ile Thr Glu Asn Lys Thr Val 245 250 255

ata gag caa cta aaa gct gat gct caa ctt aga gaa gag caa gtg cgg 816

WO 99/53948 PCT/US99/08744 Ile Glu Gln Leu Lys Ala Asp Ala Gln Leu Arg Glu Glu Gln Val Arg 265 ttt tta gaa aag cgt aaa caa gag tta gaa gag gct tgt tca aca ttg Phe Leu Glu Lys Arg Lys Gln Glu Leu Glu Glu Ala Cys Ser Thr Leu 280 tcc cat tca att gcg act cta cag gaa tcc aca acc ctt cta aag gac 912 Ser His Ser Ile Ala Thr Leu Gln Glu Ser Thr Thr Leu Leu Lys Asp 295 tct aca act aac tta cat gca gtt gaa agt cgt ctt atc ggt gtt atg 960 Ser Thr Thr Asn Leu His Ala Val Glu Ser Arg Leu Ile Gly Val Met 310 gtt cag gat ggt gca gag tcc tcc acc gta gag gaa gct tca caa gat 1008 Val Gln Asp Gly Ala Glu Ser Ser Thr Val Glu Glu Ala Ser Gln Asp 330 gat agc gcg caa ccc caa gat gaa aat caa tct gat gct gga gag cat 1056 Asp Ser Ala Gln Pro Gln Asp Glu Asn Gln Ser Asp Ala Gly Glu His 345 aaa gat agt taa 1068 Lys Asp Ser 355 <210> 8 <211> 355 <212> PRT <213> Chlamydia psittaci <400> 8 Met Thr Val Ser Thr Asp Asn Thr Ser Pro Val Ile Ser Arg Ala Ser Ser Pro Thr Phe Gly Asp His Gly Lys Asp Phe Asp Asn Asn Lys Ile Ile Pro Ile Ser Ile Glu Ala Pro Thr Ser Ser Ala Ala Ala Val Gly 40 Ala Lys Thr Ala Ile Glu Pro Glu Gly Arg Ser Pro Leu Leu Gln Arg Ile Cys Tyr Leu Val Lys Ile Ile Ala Ala Ile Ala Leu Phe Val Val Gly Ile Ala Ala Leu Val Cys Leu Tyr Leu Gly Ser Val Ile Ser Thr Pro Ser Leu Ile Leu Met Leu Ala Ile Met Leu Val Ser Phe Val Ile Val Ile Thr Ala Ile Arg Asp Gly Thr Pro Ser Gln Val Val Arg His Met Lys Gln Gln Ile Gln Gln Phe Gly Glu Glu Asn Thr Arg Leu His

wo	99/53	948												P	CT/US	99/08744
Thr 145	Ala	Val	Glu	Asn	Leu 150	Lys	Ala	Val	Asn	Val 155	Glu	Leu	Ser	Glu	Gln 160	
Ile	Asn	Gln	Leu	Lys 165	Gln	Leu	His	Thr	Arg 170	Leu	Ser	Asp	Phe	Gly 175	Asp	
Arg	Leu	Glu	Ala 180	Asn	Thr	Gly	Asp	Phe 185	Thr	Ala	Leu	Ile	Ala 190	Asp	Phe	
Gln	Leu	Ser 195	Leu	Glu	Glu	Phe	Lys 200	Ser	Val	Gly	Thr	Lys 205	Val	Glu	Thr	
Met	Leu 210	Ser	Pro	Phe	Glu	Lys 215	Leu	Ala	Gln	Ser	Leu 220	Lys	Glu	Thr	Phe	
Ser 225	Gln	Glu	Ala	Val	Gln 230	Ala	Met	Met	Ser	Ser 235	Val	Thr	Glu	Leu	Arg 240	
Thr	Asn	Leu	Asn	Ala 245	Leu	Lys	Glu	Leu	Ile 250	Thr	Glu	Asn	Lys	Thr 255	Val	
Ile	Glu	Gln	Leu 260	Lys	Ala	Asp	Ala	Gln 265	Leu	Arg	Glu	Glu	Gln 270	Val	Arg	
Phe	Leu	Glu 275	Lys	Arg	Lys	Gln	Glu 280	Leu	Glu	Glu	Ala	Cys 285	Ser	Thr	Leu	
Ser	His 290	Ser	Ile	Ala	Thr	Leu 295	Gln	Glu	Ser	Thr	Thr 300	Leu	Leu	Lys	Asp	
Ser 305	Thr	Thr	Asn	Leu	His 310	Ala	Val	Glu	Ser	Arg 315	Leu	Ile	Gly	Val	Met 320	
Val	Gln	Asp	Gly	Ala 325	Glu	Ser	Ser	Thr	Val 330	Glu	Glu	Ala	Ser	Gln 335	Asp	
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				Ala											caa Gln	96

WO 99/53948 PCT/US99/08744 gcg aaa gaa ctt gaa acg aaa gtc agt ttg gta gac aga aca gct act Ala Lys Glu Leu Glu Thr Lys Val Ser Leu Val Asp Arg Thr Ala Thr tta tca ctt acc act ggc aat aat gta gcc acg gat gta ctc ctt tta Leu Ser Leu Thr Thr Gly Asn Asn Val Ala Thr Asp Val Leu Leu Leu aaa gat gag gtt gca gaa cta aaa gga tgt ttg tct gca gtt acg gat 240 Lys Asp Glu Val Ala Glu Leu Lys Gly Cys Leu Ser Ala Val Thr Asp cta tta atc ege tca gge tca tca aga aca ect ggg ggt get eet aat 288 Leu Leu Ile Arg Ser Gly Ser Ser Arg Thr Pro Gly Gly Ala Pro Asn cca gaa ggc act aat tac cta ata gga tgc aca cct cct tct ctt tqc Pro Glu Gly Thr Asn Tyr Leu Ile Gly Cys Thr Pro Pro Ser Leu Cys 100 105 gct aaa ctt aca gcg tta gcg tta aca att ata gcc ctc att gct atc Ala Lys Leu Thr Ala Leu Ala Leu Thr Ile Ile Ala Leu Ile Ala Ile 115 120 aca gta ctt gtt atc tgt att gtt act gtt tgc ggc ggt ttc ccc cta 432 Thr Val Leu Val Ile Cys Ile Val Thr Val Cys Gly Gly Phe Pro Leu 130 135 ttt att tcc cta ctc aac atg tac aca gtt ggt gct tgt ata tcc tta 480 Phe Ile Ser Leu Leu Asn Met Tyr Thr Val Gly Ala Cys Ile Ser Leu 145 150 ccg atc att tcg tgt gcc gca gtt tca atg atg att cta tgc tca cat 528 Pro Ile Ile Ser Cys Ala Ala Val Ser Met Met Ile Leu Cys Ser His 165 tct att aac tct tta tta aga aac agg cct gcg atc tat atg act aac 576 Ser Ile Asn Ser Leu Leu Arg Asn Arg Pro Ala Ile Tyr Met Thr Asn 180 aat ttt caa aca gaa tct taa 597 Asn Phe Gln Thr Glu Ser 195 <210> 10 <211> 198 <212> PRT <213> Chlamydia psittaci <400> 10 Met Ser Thr Thr Pro Ala Ser Ser Ala Ser Arg Asp Val Leu Leu Asp Asp Val Leu Ile Ala Phe Asn Arg Lys Leu Asn Leu Val Glu Gln Gln Ala Lys Glu Leu Glu Thr Lys Val Ser Leu Val Asp Arg Thr Ala Thr 40

Leu Ser Leu Thr Thr Gly Asn Asn Val Ala Thr Asp Val Leu Leu Leu

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60

Lys Asp Glu Val Ala Glu Leu Lys Gly Cys Leu Ser Ala Val Thr Asp

55

70 75

Leu Leu Ile Arg Ser Gly Ser Ser Arg Thr Pro Gly Gly Ala Pro Asn 90

Pro Glu Gly Thr Asn Tyr Leu Ile Gly Cys Thr Pro Pro Ser Leu Cys 105

Ala Lys Leu Thr Ala Leu Ala Leu Thr Ile Ile Ala Leu Ile Ala Ile 120

Thr Val Leu Val Ile Cys Ile Val Thr Val Cys Gly Phe Pro Leu 135

Phe Ile Ser Leu Asn Met Tyr Thr Val Gly Ala Cys Ile Ser Leu

Pro Ile Ile Ser Cys Ala Ala Val Ser Met Met Ile Leu Cys Ser His 170

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Asn Phe Gln Thr Glu Ser 195

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<212> DNA

<213> Chlamydia psittaci

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caq act qtt ctc gca ggg ata caa caa caa cat cct tta aac ggt ggt Gln Thr Val Leu Ala Gly Ile Gln Gln His Pro Leu Asn Gly Gly 35

tqq cct caq cat cat cct act ggc gct gca gat caa aat tat ctc atg Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met 50

cgt ctg atg caa tct cat atg gca agt acc gta tca gca gta tct gaa Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu 65

tta aga acc gaa gtc act gca atc aag aca aaa ttg cac ggg cta tct

Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser 90 act cca gct aat gtt tgc agc ggt cct atg gct cta gcc gct ttt ctt Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu 100 105 cta gct ata tct tta gtt gcg att atc atc att gtt tta gcc tcc tta Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Val Leu Ala Ser Leu ggc ctt gca ggc ata cta cct caa gct gcc gct atc tta gtg aat aca Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ala Ile Leu Val Asn Thr gca aac tot ata tgg gct att gtt agc gct tcg ata gtc act gtt atc Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile tgc tta att agc gtg cta tgc ata acg cta att cga cac cat aaa ccc Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro 170 tta cct att gaa act agg cct acc gga cat taa 561 Leu Pro Ile Glu Thr Arg Pro Thr Gly His 180 <210> 12 <211> 186 <212> PRT <213> Chlamydia psittaci <400> 12 Met Thr Ser Val Arg Thr Asp Leu Thr Pro Gly Asp Thr Ser Leu Gln Ser Ser Leu Leu Asn Pro Ser Asp Leu Thr Thr Gln Leu Ser Asn Leu 20 Gln Thr Val Leu Ala Gly Ile Gln Gln His Pro Leu Asn Gly Gly Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu

Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser

Leu Arg Thr Giu Vai Thr Ala lie Lys Thr Lys Leu His Gly Leu Ser 85 90 95

Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu 100 105 110

Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Ile Val Leu Ala Ser Leu 115 120 125

Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ile Leu Val Asn Thr 130 135 140

Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile 145 150 155 160

Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro 165 170 175

Leu Pro Ile Glu Thr Arg Pro Thr Gly His
180 185

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<212> DNA

<213> Chlamydia trachomatis

<220>

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Ser Tyr Ser Ala Asn Arg Val Pro Gln Pro Ser Leu Met Asp Lys Ile
20 25 30

aag aaa ata gca gcc att gcc tcc cta att ctt ata ggc aca ata ggc 144
Lys Lys Ile Ala Ala Ile Ala Ser Leu Ile Leu Ile Gly Thr Ile Gly
35 40 45

ttt tta gct ctt ttg gga cat ctt gtt ggc ttt ctg atc gct cca caa 192 Phe Leu Ala Leu Leu Gly His Leu Val Gly Phe Leu Ile Ala Pro Gln 50 55

atc act att gtt ctt ctt gcc cta ttc att acc tca tta gca ggg aat 240 Ile Thr Ile Val Leu Leu Ala Leu Phe Ile Thr Ser Leu Ala Gly Asn 65 70 75 80

gct ctt tat cta cag aaa acc gct aat cta cat cta tac cag gat ctg 288
Ala Leu Tyr Leu Gln Lys Thr Ala Asn Leu His Leu Tyr Gln Asp Leu
85 90 95

caa aga gaa gtt ggg tct cta aaa gaa att aat ttc atg ctg agc gtt 336 Gln Arg Glu Val Gly Ser Leu Lys Glu Ile Asn Phe Met Leu Ser Val

cta cag aaa gaa ttt ctt cat tta tct aaa gaa ttt gca acg aca tct 384 Leu Gln Lys Glu Phe Leu His Leu Ser Lys Glu Phe Ala Thr Thr Ser 115 120 125

aaa gac ctc tct gct gta tct caa gat ttt tat tct tgt ttg caa gga 432 Lys Asp Leu Ser Ala Val Ser Gln Asp Phe Tyr Ser Cys Leu Gln Gly

130 135 140

ttt aga gat aac tat aaa ggt ttt gaa tct ctt ttg gat gag tat aaa 480 Phe Arg Asp Asn Tyr Lys Gly Phe Glu Ser Leu Leu Asp Glu Tyr Lys 145 150 155 160

aac tot aca gaa gaa atg ogo aaa oto ttt tog caa gaa ato ata goa 528

WO 99/53948 PCT/US99/08744 Asn Ser Thr Glu Glu Met Arg Lys Leu Phe Ser Gln Glu Ile Ile Ala 170 gat ctt aaa ggc tct gtt gcc tca tta aga gag gaa atc cga ttc cta 576 Asp Leu Lys Gly Ser Val Ala Ser Leu Arg Glu Glu Ile Arg Phe Leu 185 ace eca tta gea gaa gaa gtt ege ega tta geg eat aac eag gaa tea Thr Pro Leu Ala Glu Glu Val Arg Arg Leu Ala His Asn Gln Glu Ser 200 tta aca gcg gct att gaa gaa tta aaa aca att cgt gat agc tta cga Leu Thr Ala Ala Ile Glu Glu Leu Lys Thr Ile Arg Asp Ser Leu Arg 215 gat gaa att gga caa ctt tca caa ctt tct aaa act ctt acc agt caa Asp Glu Ile Gly Gln Leu Ser Gln Leu Ser Lys Thr Leu Thr Ser Gln 235 att gca tta caa cga aaa gag agc tca gat ctg tgt tcc cag ata aga Ile Ala Leu Gln Arg Lys Glu Ser Ser Asp Leu Cys Ser Gln Ile Arg gag acg ctc tcc ccc aga aag tct gca tca ccc tct aca aaa agc Glu Thr Leu Ser Ser Pro Arg Lys Ser Ala Ser Pro Ser Thr Lys Ser tcc tag 822 Ser <210> 14 <211> 273 <212> PRT <213> Chlamydia trachomatis <400> 14 Met Thr Thr Pro Thr Leu Ile Val Ile Pro Pro Ser Pro Pro Ala Pro Ser Tyr Ser Ala Asn Arg Val Pro Gln Pro Ser Leu Met Asp Lys Ile Lys Lys Ile Ala Ala Ile Ala Ser Leu Ile Leu Ile Gly Thr Ile Gly Phe Leu Ala Leu Leu Gly His Leu Val Gly Phe Leu Ile Ala Pro Gln Ile Thr Ile Val Leu Leu Ala Leu Phe Ile Thr Ser Leu Ala Gly Asn 75 Ala Leu Tyr Leu Gln Lys Thr Ala Asn Leu His Leu Tyr Gln Asp Leu Gln Arg Glu Val Gly Ser Leu Lys Glu Ile Asn Phe Met Leu Ser Val 105 Leu Gln Lys Glu Phe Leu His Leu Ser Lys Glu Phe Ala Thr Thr Ser

120

115

Lys Asp Leu Ser Ala Val Ser Gln Asp Phe Tyr Ser Cys Leu Gln Gly 130 140

Phe Arg Asp Asn Tyr Lys Gly Phe Glu Ser Leu Leu Asp Glu Tyr Lys 145 150 155

Asn Ser Thr Glu Glu Met Arg Lys Leu Phe Ser Gln Glu Ile Ile Ala 165 170 175

Asp Leu Lys Gly Ser Val Ala Ser Leu Arg Glu Glu Ile Arg Phe Leu 180 185 190

Thr Pro Leu Ala Glu Glu Val Arg Arg Leu Ala His Asn Gln Glu Ser 195 200 205

Leu Thr Ala Ala Ile Glu Glu Leu Lys Thr Ile Arg Asp Ser Leu Arg 210 215 220

Asp Glu Ile Gly Gln Leu Ser Gln Leu Ser Lys Thr Leu Thr Ser Gln 225 230 235 240

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Glu Thr Leu Ser Ser Pro Arg Lys Ser Ala Ser Pro Ser Thr Lys Ser 260 265 270

Ser

<210> 15

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<220>

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aac acc gta act att ggt gca tgc gta tcc ttg ccg gta ttc act tgc 240
Asn Thr Val Thr Ile Gly Ala Cys Val Ser Leu Pro Val Phe Thr Cys
65 70 75 80

ata get aca acg tta tta ett ett tgt ete egt aat ate gaa ete eta 288

PCT/US99/08744 WO 99/53948 Ile Ala Thr Thr Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu 90 gcc aga ccg caa gta ttt acc ctc tcc act caa ttc agc cca aca aaa Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys 100 105 cct caa gaa tag 348 Pro Gln Glu 115 <210> 16 <211> 115 <212> PRT <213> Chlamydia trachomatis <400> 16 Met Val His Ser Val Tyr Asn Ser Leu Ala Pro Glu Gly Phe Ser Gln Val Ser Ile Gln Pro Ser Gln Ile Pro Thr Ser Lys Lys Val Met Ile Ala Ile Met Thr Leu Phe Ala Leu Thr Ala Ile Ala Ala Ile Val Leu Ser Ile Val Thr Val Cys Gly Gly Phe Pro Phe Leu Leu Ala Ala Leu Asn Thr Val Thr Ile Gly Ala Cys Val Ser Leu Pro Val Phe Thr Cys 75 Ile Ala Thr Thr Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu 85 Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys 105 Pro Gln Glu 115 <210> 17 <211> 537 <212> DNA <213> Chlamydia trachomatis <220> <221> CDS <222> (1)..(537) <400> 17 atg acg tac tct ata tcc gat ata gca cac aaa tct gat att tct aat 48 Met Thr Tyr Ser Ile Ser Asp Ile Ala His Lys Ser Asp Ile Ser Asn ccc acg tct ccc gct cca tca aga aaa cga gga tcc ttt ccc cca caa Pro Thr Ser Pro Ala Pro Ser Arg Lys Arg Gly Ser Phe Pro Pro Gln

20

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Leu Cys His Ile Thr Phe Ser Leu Pro Ala Gly Ala Met Ala Ala Ile $50 \hspace{1.5cm} 55 \hspace{1.5cm} 60$

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Phe Ser Lys Ala His His Arg Ile Ala Tyr Met Pro Gln Arg Ala Ser 100 105 110

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Cys Tyr Gly Tyr Lys Gly Ile Trp Asn Arg Ile Ser Thr Asp Asp Arg 130 \$135\$

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Leu Phe Ser Ala Ile Asp Met Ala Ser Tyr Gln Met Val Val Asp Val 195 200 205

Leu Gln Glu Leu Lys Ser Glu Gly Lys Thr Ile Val Val Ile His His 210 215 220

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<220>

<221> CDS

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WO 99/53948 PCT/US99/08744 Ile Glu Gln Leu Lys Ala Asp Ala Gln Leu Arg Glu Glu Gln Val Arg 265 ttt tta gaa aag cgt aaa caa gag tta gaa gag gct tgt tca aca ttg Phe Leu Glu Lys Arg Lys Gln Glu Leu Glu Glu Ala Cys Ser Thr Leu 280 tcc cat tca att gcg act cta cag gaa tcc aca acc ctt cta aag gac Ser His Ser Ile Ala Thr Leu Gln Glu Ser Thr Thr Leu Leu Lys Asp 290 295 tet aca act aac tta cat gea gtt gaa agt egt ett ate ggt gtt atg 960 Ser Thr Thr Asn Leu His Ala Val Glu Ser Arg Leu Ile Gly Val Met 305 310 gtt cag gat ggt gca gag tcc tcc acc gta gag gaa gct tca caa gat 1008 Val Gln Asp Gly Ala Glu Ser Ser Thr Val Glu Glu Ala Ser Gln Asp 325 gat agc gcg caa ccc caa gat gaa aat caa tct gat gct gga gag cat Asp Ser Ala Gln Pro Gln Asp Glu Asn Gln Ser Asp Ala Gly Glu His 345 aaa gat agt taa 1068 Lys Asp Ser 355 <210> 8 <211> 355 <212> PRT <213> Chlamydia psittaci <400> 8 Met Thr Val Ser Thr Asp Asn Thr Ser Pro Val Ile Ser Arg Ala Ser Ser Pro Thr Phe Gly Asp His Gly Lys Asp Phe Asp Asn Asn Lys Ile 25 Ile Pro Ile Ser Ile Glu Ala Pro Thr Ser Ser Ala Ala Ala Val Gly Ala Lys Thr Ala Ile Glu Pro Glu Gly Arg Ser Pro Leu Leu Gln Arg Ile Cys Tyr Leu Val Lys Ile Ile Ala Ala Ile Ala Leu Phe Val Val Gly Ile Ala Ala Leu Val Cys Leu Tyr Leu Gly Ser Val Ile Ser Thr Pro Ser Leu Ile Leu Met Leu Ala Ile Met Leu Val Ser Phe Val Ile 100 105 Val Ile Thr Ala Ile Arg Asp Gly Thr Pro Ser Gln Val Val Arg His Met Lys Gln Gln Ile Gln Gln Phe Gly Glu Glu Asn Thr Arg Leu His 130 135

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Arg	Leu	Glu	Ala 180	Asn	Thr	Gly	Asp	Phe 185	Thr	Ala	Leu	Ile	Ala 190	Asp	Phe	
Gln	Leu	Ser 195	Leu	Glu	Glu	Phe	Lys 200	Ser	Val	Gly	Thr	Lys 205	Val	Glu	Thr	
Met	Leu 210	Ser	Pro	Phe	Glu	Lys 215	Leu	Ala	Gln	Ser	Leu 220	Lys	Glu	Thr	Phe	
Ser 225	Gln	Glu	Ala	Val	Gln 230	Ala	Met	Met	Ser	Ser 235	Val	Thr	Glu	Leu	Arg 240	
Thr	Asn	Leu	Asn	Ala 245	Leu	Lys	Glu	Leu	Ile 250	Thr	Glu	Asn	Lys	Thr 255	Val	
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Phe	Leu	Glu 275	Lys	Arg	Lys	Gln	Glu 280	Leu	Glu	Glu	Ala	Cys 285	Ser	Thr	Leu	
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Ser 305	Thr	Thr	Asn	Leu	His 310	Ala	Val	Glu	Ser	Arg 315		Ile	Gly	Val	Met 320	
Val	Gln	Asp	Gly	Ala 325		Ser	Ser	Thr	Val 330		Glu	Ala	Ser	Gln 335	_	
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gac Asp	gtt Val	tta Lev	ata 1 Ile 20	e Ala	ttt Phe	aat Ası	aga n Arg	aag Lys 25	Lei	a aat 1 Asi	t cto n Lev	gta lVal	gaa Glu 30	ı Glr	caa Gln	96

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55
60

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Lys Asp Glu Val Ala Glu Leu Lys Gly Cys Leu Ser Ala Val Thr Asp 65 70 75 80

Leu Leu Ile Arg Ser Gly Ser Ser Arg Thr Pro Gly Gly Ala Pro Asn 85 90 95

Pro Glu Gly Thr Asn Tyr Leu Ile Gly Cys Thr Pro Pro Ser Leu Cys 100 105 110

Ala Lys Leu Thr Ala Leu Ala Leu Thr Ile Ile Ala Leu Ile Ala Ile 115 120 125

Thr Val Leu Val Ile Cys Ile Val Thr Val Cys Gly Gly Phe Pro Leu 130 140

Phe Ile Ser Leu Leu Asn Met Tyr Thr Val Gly Ala Cys Ile Ser Leu 145 150 155 160

Pro Ile Ile Ser Cys Ala Ala Val Ser Met Met Ile Leu Cys Ser His 165 $\,$ 170 $\,$ 175 $\,$

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Asn Phe Gln Thr Glu Ser 195

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·<220>

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cag act gtt ctc gca ggg ata caa caa caa cat cct tta aac ggt ggt 144
Gln Thr Val Leu Ala Gly Ile Gln Gln His Pro Leu Asn Gly Gly

tgg cct cag cat cat cct act ggc gct gca gat caa aat tat ctc atg 192
Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met

cgt ctg atg caa tct cat atg gca agt acc gta tca gca gta tct gaa 240
Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu
65 70 75 80

tta aga acc gaa gtc act gca atc aag aca aaa ttg cac ggg cta tct 288

Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser 90 act cca gct aat gtt tgc agc ggt cct atg gct cta gcc gct ttt ctt 336 Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu cta gct ata tct tta gtt gcg att atc atc att gtt tta gcc tcc tta 384 Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Ile Val Leu Ala Ser Leu 120 115 ggc ctt gca ggc ata cta cct caa gct gcc gct atc tta gtg aat aca 432 Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ala Ile Leu Val Asn Thr 130 135 480 gca aac tot ata tgg gct att gtt agc gct tcg ata gtc act gtt atc Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile 145 150 155 tgc tta att agc gtg cta tgc ata acg cta att cga cac cat aaa ccc 528 Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro 165 170 561 tta cct att gaa act agg cct acc gga cat taa Leu Pro Ile Glu Thr Arg Pro Thr Gly His 180 185 <210> 12

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Gin Thr Val Leu Ala Gly Ile Gln Gln Gln His Pro Leu Asn Gly Gly 35 40 45

Trp Pro Gln His His Pro Thr Gly Ala Ala Asp Gln Asn Tyr Leu Met 50 60

Arg Leu Met Gln Ser His Met Ala Ser Thr Val Ser Ala Val Ser Glu 65 70 75 80

Leu Arg Thr Glu Val Thr Ala Ile Lys Thr Lys Leu His Gly Leu Ser 85 90 95

Thr Pro Ala Asn Val Cys Ser Gly Pro Met Ala Leu Ala Ala Phe Leu 100 105 110

Leu Ala Ile Ser Leu Val Ala Ile Ile Ile Val Leu Ala Ser Leu 115 120 125

Gly Leu Ala Gly Ile Leu Pro Gln Ala Ala Ala Ile Leu Val As
n Thr $130 \hspace{1.5cm} 135 \hspace{1.5cm} 140 \hspace{1.5cm}$

Ala Asn Ser Ile Trp Ala Ile Val Ser Ala Ser Ile Val Thr Val Ile 145 150 155 160

Cys Leu Ile Ser Val Leu Cys Ile Thr Leu Ile Arg His His Lys Pro 165 170 175

Leu Pro Ile Glu Thr Arg Pro Thr Gly His 180

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Phe Leu Ala Leu Leu Gly His Leu Val Gly Phe Leu Ile Ala Pro Gln
50 55

atc act att gtt ctt ctt gcc cta ttc att acc tca tta gca ggg aat 240 Ile Thr Ile Val Leu Leu Ala Leu Phe Ile Thr Ser Leu Ala Gly Asn 65 70 75

gct ctt tat cta cag aaa acc gct aat cta cat cta tac cag gat ctg 288 Ala Leu Tyr Leu Gln Lys Thr Ala Asn Leu His Leu Tyr Gln Asp Leu 85 90 95

caa aga gaa gtt ggg tct cta aaa gaa att aat ttc atg ctg agc gtt 336 Gln Arg Glu Val Gly Ser Leu Lys Glu Ile Asn Phe Met Leu Ser Val 100 105 110

cta cag aaa gaa ttt ctt cat tta tct aaa gaa ttt gca acg aca tct 384 Leu Gln Lys Glu Phe Leu His Leu Ser Lys Glu Phe Ala Thr Thr Ser 115 120 125

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aac tot aca gaa gaa atg cgc aaa ctc ttt tcg caa gaa atc ata gca 528

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acc Thr	cca Pro	tta Leu 195	gca Ala	gaa Glu	gaa Glu	gtt Val	cgc Arg 200	cga Arg	tta Leu	gcg Ala	cat His	aac Asn 205	cag Gln	gaa Glu	tca Ser	624
tta Leu	aca Thr 210	gcg Ala	gct Ala	att Ile	gaa Glu	gaa Glu 215	tta Leu	aaa Lys	aca Thr	att Ile	cgt Arg 220	gat Asp	agc Ser	tta Leu	cga Arg	672
gat Asp 225	gaa Glu	att Ile	gga Gly	caa Gln	ctt Leu 230	tca Ser	caa Gln	ctt Leu	tct Ser	aaa Lys 235	act Thr	ctt Leu	acc Thr	agt Ser	caa Gln 240	720
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gag Glu	acg Thr	ctc Leu	tcc Ser 260	tcc Ser	ccc Pro	aga Arg	aag Lys	tct Ser 265	gca Ala	tca Ser	ccc Pro	tct Ser	aca Thr 270	aaa Lys	agc Ser	816
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Ser	Tyr	Ser	Ala 20	Asn	Arg	Val	Pro	Gln 25	Pro	Ser	Leu	Met	Asp 30	Lys	Ile	
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gcg Ala	ata Ile	atg Met 35	Thr	ctt Leu	ttt Phe	gca Ala	ctc Leu 40	aca Thr	gcc Ala	att Ile	gca Ala	gca Ala 45	Ile	gtc Val	ctt Leu	144
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aac Asn 65	Thr	gta Val	act Thr	att Ile	ggt Gly 70	Ala	tgc Cys	gta Val	tcc Ser	ttg Leu 75	Pro	gta Val	ttc Phe	act Thr	tgc Cys 80	240
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WO 99/53948 PCT/US99/08744 Ile Ala Thr Thr Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu gcc aga ccg caa gta ttt acc ctc tcc act caa ttc agc cca aca aaa 336 Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys cct caa gaa tag 348 Pro Gln Glu 115 <210> 16 <211> 115 <212> PRT <213> Chlamydia trachomatis <400> 16 Met Val His Ser Val Tyr Asn Ser Leu Ala Pro Glu Gly Phe Ser Gln Val Ser Ile Gln Pro Ser Gln Ile Pro Thr Ser Lys Val Met Ile Ala Ile Met Thr Leu Phe Ala Leu Thr Ala Ile Ala Ile Val Leu Ser Ile Val Thr Val Cys Gly Gly Phe Pro Phe Leu Leu Ala Ala Leu Asn Thr Val Thr Ile Gly Ala Cys Val Ser Leu Pro Val Phe Thr Cys Ile Ala Thr Thr Leu Leu Leu Cys Leu Arg Asn Ile Glu Leu Leu Ala Arg Pro Gln Val Phe Thr Leu Ser Thr Gln Phe Ser Pro Thr Lys Pro Gln Glu 115 <210> 17 <211> 537 <212> DNA <213> Chlamydia trachomatis <220> <221> CDS <222> (1)..(537) atg acg tac tct ata tcc gat ata gca cac aaa tct gat att tct aat Met Thr Tyr Ser Ile Ser Asp Ile Ala His Lys Ser Asp Ile Ser Asn ccc acg tct ccc gct cca tca aga aaa cga gga tcc ttt ccc cca caa Pro Thr Ser Pro Ala Pro Ser Arg Lys Arg Gly Ser Phe Pro Pro Gln

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			gcc Ala							_						144
			ccc Pro				_		_						-	192
			aac Asn													240
			tta Leu													288
		_	ttt Phe 100			-	-		-	_					_	336
			atc Ile													384
	-		gtt Val	_	_	-					-			_		432
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_	agc Ser	taa														537
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Ser	Pro	Ser 35		val	. Gly	Ser	Leu 40		Gly	Ala	. Asn	Phe 45		Thr	Trp	
Gly	7 Pro 50		Pro	Phe	e Ph∈	Thr 55		. Pro	Val	Туг	Pro 60		Glr	Leu	ı Ala	
Ala		Glr	n Asr	n Asr	Leu 70		e Thi	Leu	ı Glr	Thr		ı Val	Ser	Ala	Leu 80	

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WU	YY/33	948												ŧ	C 1/U	599/08/4
Lys	Lys	Lys	Leu	Val 85	Gln	Ser	Ser	Gln	Thr 90	Arg	Gly	Ser	Leu	Gly 95	Leu	
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Val	Ala	Val 115	Ile	Val	Leu	Ala	Ser 120	Leu	Gly	Leu	Gly	Gly 125	Val	Leu	Pro	
Phe	Val 130	Leu	Val	Cys	Leu	Ala 135	Gly	Ser	Thr	Asn	Ala 140	Ile	Trp	Ala	Ile	
Val 145	Ser	Ala	Ser	Ile	Thr 150	Thr	Leu	Ile	Cys	Cys 155	Val	Ser	Ile	Ala	Cys 160	
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PCT09

RAW SEQUENCE LISTING PATEUR SPELICE 100. US/09/673,763

Defe 01/18/2-01 limf 10 54 58

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Output Set N:\CRF3\01182001\1673763.raw

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3 <110> APPLICANT Oregon State Univers to
      5 <129> FITTHE OF INVENTION: Nethods of use for infection-specific INCA, INCA, and
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C--> 10 <140> CURRENT APPLICATION NUMBER: US/09/673,763
C--> 11 <141> CURRENT FILING DATE: 2000-10-16
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    32 <221> NA-E/KFY CDS
    33 <222> GOCATION (1) (534)
35 <400> SEQUENCE 1
    36 atg add dag the tha the ort age tha dig ter hig ter tor end ont
     37 Met 1/8 Los Phe Leu Leu Leu Sor Law Mor Sor Leu Ser Ser Law Pro
    38 1. 5
                                   10
    40 aca tit gen get aat tot ana gge ned att gge ate git dat t'd om
41 Thr file la Ala Ser Thr Gly Mir (1: Gl. Lle vil Esp bed org
                    2.1
                                          25
    44 egg tige ita gaa hag tot ger ett gan had hat gan tot get gla itt 45 Arg Chs Loa Clu Clu Ser eta Ten Gly uzs ups clu ser eta che 46 ... 37 ... 40 ... 15
    48 gad dag itg day also can ith tot also ago atg ang ita gan qua
     49 Glu Tys Met i's Isn Cln The Ser isn Ser Wet Cly Lis Met Stu Glu
                                  55
     52 year dig for not use tal for aug ofn dua gan jab gai two ass sau
    53 Glu Jou Ser Ser Tle Tyr Ser Lys Leu Gln asp asp Asp Tyr Met Clu 54 65 70 75
                              70
     56 ggt ota toc day and goa you gno has life aga and aga ito gue ger
                                                                               288
     57 Cly Lee Ser Glu Thr Ala Ala Ala Glu Leu Arg f s Lys Phe Glu Asp
                    8.5
                                              9.6
    60 cta for goa gaa tac ade doa got caa gog cog for tac caa a a tta
     6.1 Bed Ser Ala Glu syr Ash Thr Ala Gln Gly Gln Tyr Tyr Gin tle ted
                   100
                                       105
                                                               110
     64 aac caa ayt bot iin aby cyc big caa bog nit aty gaa yab gid ash
    65 Ash Cln Ser Ash Pho Lys Arg He. Cln Was Tie Het Glu Glu Val Lys 66 120 125
    58 awa get lot yaa art gtg om att cas gas ggo ttg foa gto oft cut
                                                                               13.3
```

E

RAW SEQUENCE LISTING DATE: O1/13/2601 PAJLAT APPLICATION US/09/673,763 11mL 1/32/06

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69 Lys Ala Ser 31, 1n; Val and fle Glm Glm Gly Let Scr Mal ie, Lou
70 30 175
                                               140
72 aac gua jat uit gic tha het aho gut agi hog goa dat aaa uns dat
                                                                       A 30
73 Ash Clu isp ile Vol (ou sec the Asp Sor Ser Al) Asp ile Ph. Asp 74 145 150 150 155 150
                                        155
76 get sti alt ada git ell dat git ett tie daa ata att ade atg ega 77 Ala Val Ile i's Val te: Aso val led rie 178 Ile i's isn het Arg
                                                                       533
78 105 170
80 agc tag
                                                                       531
81 Ser
84 <210> SEQ 1D WO 2
85 <211> TENGIR 177
86 <21.2> TYPE PRI
87 <213> ORGANISM Chlamydia trachomatis
89 <400> SEQUENCE 2
90 Met lys Lis Phe Len len len Ser Len Met Ser Len Ser Ser len Pio
91 1 5
                                     1.)
93 Thr Pho Ala Ata Ash Sor thr Gly The fle Gt, fle Val Ash Lou Arg
94 20 25
96 Arg C/s Leu Gl: Glu Ser Ala Leu Gl. L/s Lys Glu Ser Ala GLu Phe 97 - 35 - 40 - 45
99 Glu Lys Act Lys Asi Gin Phe Ser As: Ser Me: Gly Lis Me: Glu Gitt 100 - 50 60
102 Glu Lou Scr Ser Fle Tor Ser Lys Lou Cli Asp 35p Asp Tyr Net G'u
103 65 70 75 80
105 GLz Leu Ser Glu Inc Ala Ala Ala Clu Chu teu Arg Iys Lys Phe Glu Asb 106 -90 95
108 Leu Ser Ala Ciu Fyr Asn Thr Ala Cin Gly Gin Ty: Tir Gin the leu 109 $100$
11.1 Ash Gln Sar Asa Phe L,s /rg Met Gln Lys 11c Het Gln Gh, Va) L/s 112 175 12\%
114 Lys Ala Ser Giu Thr Val Arg The Gin Glo Gli Lei Ser Val Le. Leu
115 130 135 140
117 Ash Giu Asp (ie vul Leu Ser ile Asp Ser Ser Ala Asp fyd il) asp 118 145 $150$ $150$ $155$
120 Ala Val Ile Lys Val Leu Asp Val Teu Phe Lys Ile Tio Ash Fot Arg
121 165
                                     170
123 Ser
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133 <222> LOCATION. (1) (846)
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137 Met Asn Ary Met 11e Cys Asp Cys Val Ser Arg Ile for Gly Asp arg
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140	gte	aag	Jal	at t	glit	ctg	att	Gul	aga	900	611	gu,	dat	cat	cca	tat	96
141	Val	$L\gamma S$	Asn	10	Val.	Led	-1e	Asp	Gi,	Ala	' ' e	ASP	Pro	his	Sor	Ivr	
142				20				-	25			•		30		4	
144	gag	atg	qtq	aaq	aga	dat	ada	ത്വദ	caa	at.c	ac.	elc.	dan			att	141
145	Glu	net	Val	L''S	Gĺs	ÉSO	dla	aso	Arg	Het	23.6	(to:	510	Gi a	Con	16	• •
146			35		-	-		40	,				4.5	0 ,,	(1		
	111	Lge	63!	ant	tla	cal	1 - 4		z ni	800	, cost			co. et		c > t	192
149	Pho	Cys	Zen	911	. Oli	and t	f Au	214	L.	0, 2	7	Cox	3.00	S W.A	14.0	CGC	19.
150		50	7,511	(1)	1.6-7	(), }	35	010	15	3(1	~ . C.	50	E Agric	F 1 9	1.5	515	
	01.5	gag	, + , + +	126	(3/3.)			/r1 r		110					- 1 1		
152	Lou	71	990	aac	Dina	ana	9.0	4.2	yar	' La	391	- rau	rig	119	CLE	aac	210
154	65	Clu	· ·	. 511	200	70	, d.,	الماليان الأسا	(151)	TF, (*	411	, l t	e.rc	LOL	Leu		
		2.10									, ,					80	
		acc															238
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158					8.5					1.3					+3		
160	att	taa	acg	gut	ato	वन्त	gta	tgg	व्यट	10,	act	gta	ते हे त	वसव	at g	agr	336
	110	i,tb	fhr		ket	Arq	Val	l,r io		Vr , 3	Aa	Vall	L/S	Glu	ne.	Ala	
162				100					105					110			
1.64	geq	उटव	tta	att	caa	Qua	1 11	cct	(dð	411	الاداط	gau	gal	177	262	aag	381
165	Ala	Ald	Leu	176	Gln	Gin	Phe	Pro	Gln	1,1	Glu	$\sigma_{\rm cu}$	Asp	المهاردا	Gln	L∨s	
1.66			115					120					120				
1.68	aal	acá	ya:	lag	a-c	ı.ta	tea	gad	at.	gag	Gau	CIE	qat.	agt	Lgq	gra	432
169	Asn	λla	Aso	Cli	1.0	Lead	Ser	Clu	Fet	Sta	G1.4	fou	Asp	Arσ	Trp	Ala	
170		130					L35					140			•		
172	gtg	cgt	tet	cto	tct	ace	att	cet	qaa	. તત	aa+	007	tat	t.Fa	artic	aca	189
		Viā															
	145	-				150					155	- ,	-2.			160	
176	adc	cac	aa+	acq	tild	aut	tac	t-t	ict.	cat	ctra	ta'	cta	TCC	tot		538
		His															3.50
178	-				155					170	,	2 1 2		0.47.2	1.2.5	11110	
	aca	gag	aua	ata		ggr	(13.8	than	ens.		cat	1.0	AF.	tor		ca.	5~6
181	Ala	Clu	Ara	V	Ser	GIV	(2.3)	Trn	A. M	Sar	220	200	T10	35.0	Dro	qua.	2 0
182		0.70	7.2. 9	180	1761	01)	(12.0	1-12	185	, ,	7 1 45	' ' '	LIT	190	PLO	G·u	
	dou	tiq	+ 64+		CEDIT	act	Z12 G	51.1		- 7 /7	,	/ra -	~++		0.11	** 0	(),
185	2014	Len	200	Tan O	21	3 . 3	cag	1.	ayı	U L.i	200	3 (12)	330	ang	103 -	y a	631
186	CALL	200	135	2.0	C-1.1	2014	(3.1.11	200	Ser	110	1.3	180351	205	TIC.	71. û	\ cl L	
	at.	01.101		250	+ 0.4												600
100	Arria.	quq	tat	all	CO.	gea	dat.	34.	gta	"ેલ	3. '-	grid	TEET	TLd	gad	oat	673
190	Val	G14 2:0	1 - 1	T F + 3	961	1 Ld		ADD	144	ULU	N.O. T.		Pne	Tean	G1u	", SD	
	200		1				215					230					
		rta															720
		Leu	~SII	GIT	ASD		767	Arg	1.78	LIC		ser	1,7,8	Ser	l,∨s		
194						230					255					240	
		caa															768
	GIY	$G \perp n$	175	lie		∟، د	Ala	Lys	Ser		Tent	$I \ni \mathcal{L}$	Ser	⁄sp		Val	
198					215					520					255		
200	tgt	Gort	aac	tat	ttt	age	arq	t-c	cay	cac	વેલે	44.	age	∂Са	art	30d	816
	Cys	Asp	$\mathbb{A} \in \mathbb{N}$		Phe	scr	Thr	Phe	$C \vdash n$	Bis	Ash	Val	Arg	rhr	1 ± 0	Ihr	
202				260					265					270			
204	gaa	gaa	Llg	gga	ggg	act	gt.t	elt	gau	1 ag							840

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209 <210 SEQ 1D NO 4
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211 <212> TYOF PR.
212 <213> ORGANISH Chiamydia trachometra
214 <400> Shourage
215 Mer. Ash and her the Cys asp Chs Mal Ser Aig the Lot Chy Asp Aig
216 1 5 10 15
218 Val Lys Ash lie Vul Led lie asp Clv Ala lie Asp Pro his Ser Tyr 219 $20$ . 35
221 Gla Met Val bys GIV Asp C u Asp Ard het Ala hot Sor Gla lou 17e
222 - 35 - 40 - 45
224 Pho Cvs Ash Gly lou Ci/ Tou Gru His Ser Ald Ser Leu Ard Lys His 225 50 60
227 hen Giu Gir Ask Pro 1,5 Val Val Asp len Gir om Arg fou hot Ash
228 65 70 75 80
230 Lys Ash C/s Pho Asp led Lou Sor Giu Glu Giy Phe Pro Asp Pro Als 23.1 $85\, 95\,
233 The 1rp thr Asp Jet Arg Val frp Giv Ala Ala Val Lis Glu Ket .la 234 ^{\circ} 100 ^{\circ} 105
245 Glv His /sn Ma Phe Ser Pyc Pho Thr Aig Arg Lyr Lei Ser Ser Asp 246 165 170 175
248 Aia Glu Arg Val Ser Gly Cla Tro Ard Ser Arg Cys ile Ser Aro Cru 249 $\rm 3.0^{\circ}$
251 GIV her Ser Pto GL, fix Gir the Sec 11e Atd Asp The Het \murg Val 252 \pm 105 \pm 200 \pm 205
254 Val Glu Per lie Scr Ala Ash Asp Val Glu Pal Val Phe let Glu Asp 255 - 210 - 275 - 220
257 Thi leu Asr Jin Asp Aid Leu Arg Lys IIe Val Sor Cys Sor Lys Sor 258 225 230 230 230
260 Gly Glu 175 l.c Ar, new Ala Lys Sor Pro Lou Tyr Ser Asp Asr Vol. 261 250 255
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 Dr. %
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 US/09/670,763
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			S Z E. EQUEL		(-) 3	(0)	51)										
							4 - 1	croa	sqt	F	sac	1000	.a + /r	rtc	aca	caa	48
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282	1				5	3 22 1	, , ,,	, , ,,		10	U.S.	1		. 110	3.5	G I II	
284	tta	cag	ટકવ	uat	t.cra	ana	ача	ct.a	utc		aat	00%	gal	de.1		art	96
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286				20	,				23			,	- 1	30			
288	get	Liga	tic	gta	gaq	qı.	cit	tq-	grt	aut.	tar	aut	cac	tca	gari	ate	144
									Val								
290			35					40				-	15				
292	t.t.a	ţur	CdC	a*+	ant	rtt	100	CLJ	cct	J.C.	ggg	gida	atg	get	00	ot.	190
									Pro								
294		50					25					60					
									aat								240
		Cly	1,10	$\ell \supset \mathbb{N}$	617		급분기	lvs	Ser	iliz	$ge_{n^{\perp}}$	LCL	1.78	Ala	Ser	Leu	
	65					70					75					80	
									caa								368
	$GL_{\mathcal{F}}$	71651	1 [6	Ara		Se 1	Ser	Clar	$G_{1}D$		I eu	Dyt.	Pae	GIV		Frg	
302		.			8.5					9.0					95		
									CCC								336
306	P.W.	2, 1	⊐, S	100	H LS	415	F1 9	116	λJa	1.1	116.1	5 TO	GLi		AIG	SCL	
	41.75		+ 00		110		2 + 0	vat	105 gLt	0.1		at a	4	110			10.4
									Val								384
310	, at	,,,,,	113	336	110	.,0	inc c	120	V (A 1	L 5-11	~ap	1 4-61	±25	me a	1.01	317	
	tat	tac		1.01	6.34	gaa	er a		aat	col	251	+,50		crat	gat	cot	432
									asn								1 .2
314		.50					135			5		140		p	r L p		
316	cag	quq	get	atg	cyt	att	t t.a	qao	CCA	Jt.	395		વુત્રુવ	yet	ttt	gra.	180
									210								
318						130					155					160	
									aga								528
	Asn	\sim tq	$\leq r u$	J 13		Lys	iou	Ser	Cly		G.n	Cln	Cln	Arg	$A_{+}a$	ьрь	
322					165					170					175		
324	tta	d'ad	cgq	fina	tra	arg	caa	ದರಿಡ	gca	gat	ftg	tat	ctc	atq	gat	qag	576
	Leu	Ala	Arq		ı en	Met	СПп	Lys	Ala	4SD	l e ti	LÀL	r.eu		Asp	Glu	
326				180					18=					190			
320	CLG	2.4	tide	srg.	alc	gat	वाव	gee	cot	7 37	Caa	at q	gtt	gta	9 11.	gtt	621
330	ren	Phe	195	Ala	116	asp	W_{ef}	200	ser	The	Gin	not	205	Val	2sp	Val	
	++	/1) 1	-	, 1 ± ±	22.	200	(*) 3		200	2.24		er = 01		\+ +	a +		672
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334		210	- Lu	غاناند	- 10	J C 2	215	OI/	± 7 ⊅	- 1-1	T 1 %	220	val	TIC	nis	1115	
	gat.		ani	aat	ate	Cau		ctt	r t	gal	Cat		at!	tta	110	4.0 5	720
									Phe								120
338						230				- r-	235					240	
		ca:	ctl	gLg	tac		gga	age	gua	gua		tge	t La	act	aaa		768
									Val								
												-			-		

VERIFICATION SUMMARY

TA "ENT APPLICATION US/09/673,763

DALA 01/13/2001 1555 10 34 39

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L:10 M:270 % Current Application Number differs, Replaced Application Number L:11 M:271 C. Current Filing Date differs, Replaced Current Filing Date

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled CHLAMYDIA PROTEINS AND THEIR USES, the specification of which \boxtimes is attached hereto. was filed on _____ as Application No. _____. was described and claimed in PCT International Application No. ______, filed on ______. and was amended on _____ (if applicable). \Box with amendments through ____ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application. I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed: Prior Foreign Application(s) Priority Claimed (Day/Month/Year Filed) (Number) (Country) I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below: 60/082,438 20 April 1998 60/082,588 21 April 1998 60/086,450 22 May 1998 Application Number Filing Date

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US99/08744	20 April 1999	Published		
(Application No.)	(Filing Date)	(Status)		

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from ______ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

Customer Number

-OR-

Registered practitioners listed below:



Name	Reg. No.	Name	Reg. No.
BECKER, Mark L.	31,325	OPP Dovid E	44.000
-	,	ORR, David E.	44,988
CALDWELL, Lisa M.	41,653	PETERSEN, David P.	28,106
DeGRANDIS, Paula A.	43,581	POLLEY, Richard J.	28,107
GEORGE, Samuel E.	44,119	RINEHART, Kyle B.	47,027
GIRARD, Michael P.	38, 467	SCOTTI, Robert F.	39,830
HARDING, Tanya M.	42,630	SIEGEL, Susan Alpert	43,121
JAKUBEK, Joseph T.	34,190	SLATER, Stacey C.	36,011
JONES, Michael D.	41,879	STEPHENS Jr., Donald L.	34,022
KLARQUIST, Kenneth S.	16,445	STUART, John W.	24,540
KLITZKE II, Ramon A.	30,188	VANDENBERG, John D.	31,312
LEIGH, James S.	20,434	WHINSTON, Arthur L.	19,155
MAURER, Gregory L.	43,781	WIGHT, Stephen A.	37,759
NOONAN, William D.	30,878	WINN, Garth A.	33,220

Address all telephone calls to Tanya M. Harding, Ph.D. at telephone number (503) 226-7391.

Address all correspondence to:

KLARQUIST SPARKMAN CAMPBELL LEIGH & WHINSTON, LLP One World Trade Center, Suite 1600 121 S.W. Salmon Street Portland, OR 97204-2988

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full Name of Sole or first Inventor:

Daniel D. Rockey

Inventor's Signature

lidit

1.0/12/00 Date

Residence:

Corvallis, OR

Citizenship:

United States of America

Post Office Address:

3625 NW Jackson Street Corvallis, OR 97330

Full Name of Second Inventor:

John P. Bannantine

Inventor's Signature

Residence:

Ames, IA

-

Citizenship: United States of America

Post Office Address: 903 Yuma Ave.

Ames, IA 50014